

dimensions of this cylindrically shaped Pt₁₉ core are 11.1 Å in length and 7.8 Å in diameter. The diameter is similar to that reported⁷ for catalytic platinum crystallites on alumina support.

The pseudofivefold rotational symmetry exhibited by the [Pt₁₉(CO)₁₂(μ₂-CO)₁₀]⁴⁻ tetraanion is of prime importance in that such noncrystallographically allowed symmetry has been experimentally found in a number of microcrystalline materials^{2a,8a} (e.g., vapor-grown metal whiskers of Ni, Fe, and Pt,^{8b} electrodeposited copper dendrites,^{8c} nickel grains from thermally decomposed nickel tetracarbonyl,^{8d} vapor-deposited Au on various substrates,^{8e} and synthetic diamonds^{8f}). Bagley⁹ has proposed two contact-sphere modifications for the fivefold pseudosymmetric Melmed-Hayward cork-ball structure^{8b,10} which involves the continued packing of hard spheres in concentric pentagons about a central pentagonal bipyramidal nucleus. Not only does a fragment of this Melmed-Hayward structure geometrically correspond to the Pt₁₉ core but also the dimensions of the two outer crystallographically identical pentagonal bipyramids in the Pt₁₉ core are consistent with those of the second Bagley model^{9b} based on maximum density (minimum volume) in that the Pt(A)-Pt(C)/Pt(B)-Pt(B) ratio of 2.68 Å/2.87 Å = 0.934 is close to his calculated ratio of 1.000/1.018 = 0.982.

We are currently pursuing structural characterizations of the other brown platinum carbonyl compounds in that we expect the tetraanion to have important stereochemical implications in the modeling of heterogenous metal catalysts, especially small metal aggregates dispersed on various supports.

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- (4) (a) [NBu₄][Pt₁₉(CO)₁₂(μ₂-CO)₁₀]₄·8CH₃CN: fw, 5341.1; orthorhombic; a = 17.380 (9), b = 21.201 (4), c = 16.728 (8) Å; d_{calcd} = 2.88 g cm⁻³ for Z = 2; F(000) = 4764; μ = 217.45 cm⁻¹ for Mo Kα radiation. Systematic absences of {h₁h₂0} with h₁ + h₂ = 2n + 1 indicate P2₁mn, Pm2₁n, and Pmmn as probable space groups. The latter centrosymmetric group, which was ultimately selected on the basis of least squares of trial models, conformity of intensities to hypercentric statistics, and analyses by direct methods under all three symmetries, imposes C_{2v} crystallographic site symmetry on two tetraanions. The bridging carbonyl peaks were all found from Fourier and difference Fourier maps. Anisotropic least-squares refinement of the Pt atoms with fixed carbonyl contributions gave R₁(F) = 16.9 and R₂(F) = 17.5% for 803 independent reflections (with I > 2σ(I)) which were corrected for absorption. A second data set was collected for the [n-Bu₄N]⁺ salt in which solvent was included in the capillary to prevent decay. The systematic absences and crystal system are the same with a = 16.800

(4) Å, b = 21.727 (6) Å, and c = 17.386 (6) Å for which a least-squares refinement gave R₁(F) = 13.1 and R₂(F) 17.5% for 1111 independent absorption-corrected reflections with I > 2σ_i. This model contains only the Pt atoms and did indicate a disorder of the axial carbonyls. (b) [(C₆H₅)₄P]₄[Pt₁₉(CO)₁₂(μ₂-CO)₁₀]₄·4CH₃CN: fw, 5844.8; triclinic; a = 17.234 (3), b = 25.480 (5), c = 17.171 (3) Å; α = 91.69 (2), β = 118.10 (1), γ = 74.60 (1)°; V = 6372 (2) Å³; d_{obsd} = 3.038 (4) vs. d_{calcd} = 3.046 g cm⁻³ for Z = 2; F(000) = 2594; μ = 201.1 cm⁻¹ for Mo Kα radiation. ω-Scan data were collected for the shell with 3° ≤ 2θ ≤ 40° in which 12 443 measured reflections yielded 7540 maxima with I ≥ 2σ_i. In addition, another 17 828 data were examined in the shell with 40° ≤ 2θ ≤ 55°; however, if a fast prescan did not indicate sufficient intensity for the reflection to be statistically meaningful, the reflection was not examined further. This procedure resulted in the intensity measurement of 1711 data, of which 1654 maxima possessed I > 2σ_i. A merging of the absorption-corrected data (vide infra) gave 13 515 independent data with 8596 having I > 2σ_i. Approximately 20% of the observed data were from the 40–55° shell. Thirty psican reflections, spaced approximately every 2 degrees in 2θ, were used for an empirical absorption correction. Of the 634 reflections for which a Friedel mate was collected, there were none which had intensities of the pairs differing by more than 5σ_i after the absorption correction was applied. Full-matrix least-squares refinement was employed with anisotropic thermal coefficients for the Pt and P atoms and isotropic temperature factors for the carbonyl atoms; the C₆H₅ rings were each refined as a rigid body in accord with their well-known D_{6h} geometry with individual isotropic temperature factors being varied for the carbon atoms and with the isotropic temperature factor for each hydrogen atom fixed at a B value of 2.0 greater than that of its attached carbon atom. The acetonitrile molecules were fixed. Owing to the size of the matrix and a computer limited to 265k words, all of the parameters could not be varied at one time, but instead the cluster was varied with the remainder fixed and then the cations were varied (phenyls as rigid groups). One complete cycle (both anion and cation) required ~14 computer hours when only the observed data were used. (The departmental computer is a Harris/7 with a 600-ns cycle time.) Final refinement of the 8596 independent data, with 576 parameters being varied in two blocks of full-matrix least squares, converged at R₁(F) = 9.1%, R₂(F) = 8.0% with an error of fit of 1.23 for 576 parameters.

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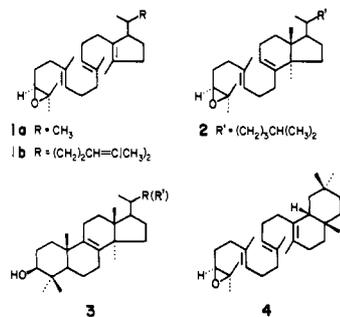
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Generation of the Onocerin System by Lanosterol 2,3-Oxidosqualene Cyclase—Implications for the Cyclization Process

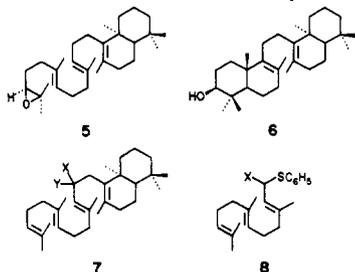
Sir:

Despite considerable structural differences between epoxides **1a**, **1b**, and **2** on the one hand and the natural substrate on the other, the enzyme lanosterol 2,3-oxidosqualene cyclase still serves to convert these substances into members of the lano-



sterol family (3).^{1,2} Likewise, the cyclase from *Pisum sativum* effects transformation of the unnatural epoxide 4³ to the same product, β -amyrin, formed from the natural progenitor,⁴ 2,3-oxidosqualene. In light of these results, there arose the question: could one of these cyclases act on a substrate candidate modified so drastically that the normal metabolic type cannot be formed? We have now answered this question with the finding that lanosterol cyclase does induce cyclization of the bicarbocyclic epoxide 5, but only as far as tetracycle 6, a representative of the structural class to which the naturally occurring plant product onocerin also belongs.

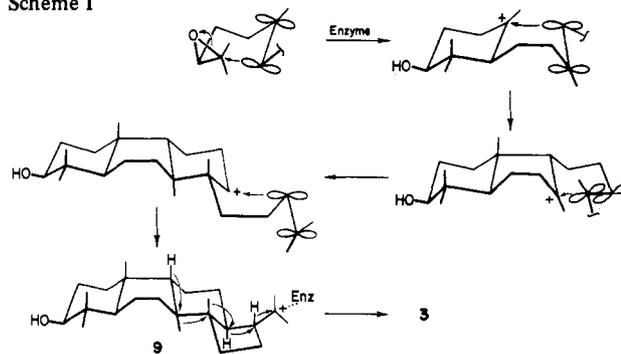
Radiolabeled *trans,trans*-(+)-tetraene 7, the precursor of 5, was obtained by modification of a previously described



route.⁵ After metalation of thioether 8 (X = ¹H) (*n*-C₄H₉Li-THF), an approximately equivalent amount of tritiated water was added, giving 8 (X = ³H) in situ. As previously described,⁵ the sulfide anion resulting from further treatment with *n*-C₄H₉Li was alkylated with (+)- Δ^8 -*trans*-bicyclopentyl bromide,^{5,6} yielding (+)-7 (X = ³H; Y = SC₆H₅). Li/C₂H₅NH₂ reduction provided 7 (X = ³H; Y = ¹H), and the latter, after successive treatment with NBS (THF-H₂O) and K₂CO₃ (MeOH), gave radiolabeled (9 × 10⁴ dpm/ μ g) (+)-5 presumably along with the second, expected racemate, which mixture was used directly in the incubation experiments.

Each preparative incubation was run with 200 μ g of radiolabeled oxide 5, which was treated successively with three 7-mL aliquots of rabbit liver enzyme solution⁷ over a 90-min period. A boiled enzyme preparation served as a control, and a squalene oxide incubation was performed concurrently to verify the enzyme activity. After denaturation of the reaction mixture with 10% methanolic KOH and extraction with ether (80–90% recovery of radioactivity), preparative TLC on silica gel (ethyl acetate-benzene) separated unchanged epoxide (*R_f* = 0.70) from products (*R_f* = 0.45–0.60, ~10% crude corrected yield). This material was further subjected to LC on a 4.5 mm × 100 cm Corasil II column using THF-hexane (2.5:97.5), giving a major peak at *T_r* = 1.50 relative to lanosterol. A portion of this enzymic product, A, was subjected to GLC on 3% OV-17 (*T_r* = 1.20 relative to lanosterol), and final purification was effected by LC on a 4.5 mm × 25 cm μ -Porasil column: NMR (100 MHz, CDCl₃) δ 0.79 (s, -CH₃), 0.82 (s, -CH₃), 0.87 (s, -CH₃), 0.96 (s, -CH₃), 0.99 (s, -CH₃), 1.24 (br s, -CH₂-), 1.65 (s, allylic -CH₃), 1.99 (br m, allylic -CH₂-), 3.26 (m, -CH₂OH); GC-MS *m/e* 426.38304 (C₃₀H₅₀O, M⁺), 408 (C₃₀H₄₈), 222 (C₁₅H₂₆O), 205 (C₁₅H₂₅, base peak). The data led to the surmise that A is a monohydroxylic β -onocerin type,

Scheme I



and, accordingly, 3 β -hydroxy- β -onoceradiene (6) was prepared by cyanoborohydride reduction of β -onoceradiene⁹ mono-*p*-toluenesulfonylhydrazone. The GLC retention times (3% OV-17) and the 100-MHz NMR spectra indicated the identity of tetracycle 6 and enzyme product A, and the high-resolution GC-MS of the two substances were superimposable. Finally, cocrystallization of 1.0 mg of authentic 6 with A (9.0 × 10⁵ dpm) provided a mixture (900 dpm/ μ g) which was thrice recrystallized from acetone-THF, giving successive specific activities of 720, 670, and 650 dpm/ μ g, thus confirming the structure as well as the relative and absolute stereochemistry of A as 6.

Previously described unnatural substrates for oxidosqualene cyclases all possess the basic carbon framework—either acyclic or partially precyclized (1a–b, 2, and 4)—needed for the natural polycyclic nucleus¹⁰ and thus were of little use in deciding whether pan-molecular control by the enzyme is a prerequisite for its action. On the basis of the results herein, a full “pocket fit” does not appear to be essential for either substrate recognition or cyclization initiation, and, in keeping with previous indications,¹ the minimal requirements for cyclase action seems to be a specifically constituted epoxide terminus and two appropriately sited π bonds. Thus, it is clear that overall, rigid maintenance of substrate and entirely concerted production of tetracycle by mammalian cyclase are not obligatory, a finding in keeping with the previously stated conclusion¹¹ that the epoxide cyclization process is stepwise, involving partially cyclized, conformationally rigid carbocationic intermediates (Scheme 1). As the experiments with 1a–b, 2, 4, and 5 suggest, formation of an enzyme-substrate complex¹² (9) at C-20 is not a requisite part of the cyclization process, but may serve to protect and store the C-20 cation preparatory to the ensuing CH₃/H migrations which lead to final product.

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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 glycylamide 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·H₂O 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 *P*2₁, 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 ($\sum ||F_o| - |F_c|| / \sum |F_o|$) 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-CH₂-THF was prepared from (±)-5,10-CH₂-THF by the enzymic depletion (thymidylate synthetase)⁵ of the natural diastereomer, (+)-5,10-CH₂-THF. This material was converted into (-)-

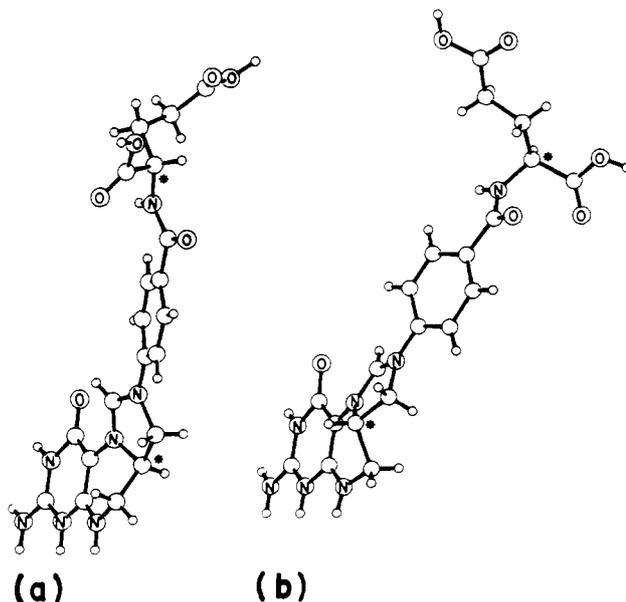


Figure 1. Structures of the N-1-protonated derivatives of (a) (+)-(5,10-CH-THF)⁺, the natural diastereomer, and (b) (-)-(5,10-CH-THF)⁺, the unnatural diastereomer. The asymmetric carbon atoms are designated by asterisks. This drawing was prepared by use of the computer program ORTEP.⁴

(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 *P*2₁, 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.

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