consistent with results of Ford[8b] for the kinetics of PPh_3 exchange with $[PPN][HRu_3(CO)_{11}]$.[8] On the basis of Ford's work,[8b] intramolecular CO exchange is probably stereoselective. Since intermolecular CO exchange is slower than intramolecular scrambling of CO's in the cluster, we employ a statistical correction factor of $^1/_{11}$ in the rate calculation.

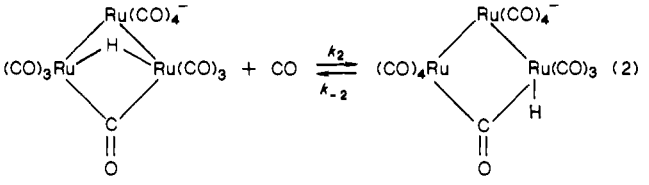
The associative exchange pathway becomes increasingly significant (reaction 1) with increasing ^{13}CO concentration (increasing ^{13}CO pressure).

$$[HRu_3(^{12}CO)_{11}]^- + {}^{13}CO \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [HRu_3(^{13}CO)(^{12}CO)_{11}]^- \quad (1a)$$

$$[HRu_3(^{13}CO)(^{12}CO)_{11}]^- \underset{k'_2}{\overset{k'_{-2}}{\rightleftharpoons}} [HRu_3(^{13}CO)(^{12}CO)_{10}]^- + {}^{12}CO \quad (1b)$$

$$[HRu_3(^{12}CO)_{11}]^- + {}^{13}CO \rightleftarrows [HRu_3(^{13}CO)(^{12}CO)_{10}]^- + {}^{12}CO \quad (1)$$

The following steps have been proposed in the reaction of $[HRu_3(CO)_{11}]^-$ with CO and H_2O in the water gas shift reaction:[1,2]



(2)

$$[HRu_3(CO)_{12}]^- + H_2O \xrightarrow{k_3} Ru_3(CO)_{12} + H_2 + OH^- \quad (3)$$

$$[HRu_3(CO)_{11}]^- + H_2O + CO \xrightarrow{k_4} Ru_3(CO)_{12} + H_2 + OH^- \quad (4)$$

The catalytic cycle is completed by reaction of the $Ru_3(CO)_{12}$ with OH^- to regenerate $[HRu_3(CO)_{11}]^-$.

Reaction 1a of the exchange pathway from the kinetic results is consistent with suggested reaction 2. Exchange of ^{13}CO with ^{12}CO in the study of ^{13}CO exchange in the deuteriated cluster $[PPh_4][DRu_3(CO)_{11}]$ shows that the deuterium label has little effect on k_1 (Figure 1) but that k_2 decreases significantly: $k_2(H)/k_2(D) = 1.40$; $k_1 = 0.247 \pm 0.005$ s^{-1}; $k_2 = 0.387 \pm 0.010$ M^{-1} s^{-1}. The dominant isotope effect[9] is consistent with our suggestion[1a] that in the associative step bridge-hydrogen displacement to a terminal position occurs. Since the intermediate does not reach detectable concentrations in the reaction medium, we invoke the steady-state approximation and set the rate constant for H_2 liberation, k_4, equal to $k_2k_3/(k_{-2} + k_3)$.

The value of k_4 is estimated to be about 1.3×10^{-3} M^{-1} s^{-1} for the liberation of H_2 from an aqueous solution 0.01 M in $K[HRu_3(CO)_{11}]$, 25 °C under 1 atm of CO[1a] (eq 3), with [CO] equal to its solubility in water.[10] Unless k_2 is subject to major solvent effects, the low value of k_4 compared to k_2 implies that $k_{-2} >> k_3$, i.e., $k_4 \sim k_2k_3/k_{-2}$. Therefore, reaction 3 approximates a preequilibrium step prior to rate-limiting release of H_2 in the second step. The rate of HD evolution from the reaction of $[DRu_3(CO)_{11}]^-$ with H_2O under 1 atm of CO is significantly smaller[1a] than the rate of H_2 evolution from the reaction of $[HRu_3(CO)_{11}]^-$ with H_2O. Thus the kinetic isotope effect on the overall reaction is larger than the kinetic isotope effect found for reaction 2, and an additional contribution from k_3 is thereby implied as expected for the making of an H–H (H–D) bond accompanied by the breaking of a Ru–H (Ru–D) bond.

The rate of ^{13}CO exchange with ^{12}CO in $[HOs_3(CO)_{11}]^-$ also obeys the overall forward rate given by eq A (Figure 1). For $[PPh_4][HOs_3(CO)_{11}]$ at 298 K, $k_1 = 0.0212 \pm 0.0010$ s^{-1} and $k_2 \sim 0.04$ M^{-1} s^{-1}. For k_1, $\Delta H_1^{\ddagger} = 23.9 \pm 0.7$ kcal/mol and $\Delta S_1^{\ddagger} = 13.9 \pm 2.3$ cal/mol K.

For $[HOs_3(CO)_{11}]^-$, the rate of exchange is relatively insensitive to ^{13}CO concentration. This poorer ability to participate in an associative reaction, we believe, accounts for the lower activity of $[HOs_3(CO)_{11}]^-$ than that of $[HRu_3(CO)_{11}]^-$ in the catalysis of the water gas shift reaction.

For the exchange of ^{13}CO with ^{12}CO in $[PPh_4][DOs_3(CO)_{11}]$, the value of k_1 is essentially unaffected. For $[PPh_4][DOs_3(CO)_{11}]$ at 298 K, $k_1 = 0.0211 \pm 0.0015$ s^{-1} and $k_2 \sim 0.03$ M^{-1} s^{-1}.

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Chirality of Intermediates in Thiamin Catalysis: Structure of (+)-2-(1-Hydroxyethyl)-3,4-dimethyl-5-(2-hydroxyethyl)thiazolium Iodide, the Absolute Stereochemistry of the Enantiomers of 2-(1-Hydroxyethyl)thiamin, and Enzymic Reaction of the Diphosphates

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The decarboxylation of pyruvate is catalyzed by enzymes which utilize thiamin diphosphate (TDP) as a cofactor.[2] The enzyme-bound covalent adduct of TDP and pyruvate loses CO_2 and is protonated to form the adduct of acetaldehyde, 2-(1-hydroxyethyl)thiamin diphosphate (HETDP).[2,3] Although TDP, the substrates, and products are achiral, the intermediates are chiral with the stereocenter at the carbon atom derived from C2 of pyruvate.[4] Optically active HETDP has been isolated from pyruvate dehydrogenase[5] and 2-(1-hydroxyethyl)thiamin (HET) has been resolved.[6,7] The absolute stereochemistry of the materials is unknown. We now report the unambiguous determination of the absolute stereochemistries through X-ray crystallographic analysis of a derivative and the reaction of each enantiomer of HETDP with pyruvate decarboxylase.

2-(1-Hydroxyethyl)thiamin (HET) was prepared and resolved as the 1:1 salt of (−)-2,3-dibenzoyltartaric acid.[7] The HET released by HCl treatment of the salt is optically active: (+)-HET ($[\alpha]^{25}_D$ +12.5° ± 0.1°). The salt of HET and (+)-2,3-dibenzoyltartaric acid was also prepared and treatment with HCl released (−)-HET ($[\alpha]^{25}_D$ −12.5 ± 0.1°). (+)-HET was converted to (−)-2-(1-hydroxyethyl)-3,4-dimethyl-5-(2-hydroxyethyl)thiazolium iodide ((−)-HETI) by reaction with sodium sulfite[8,9]

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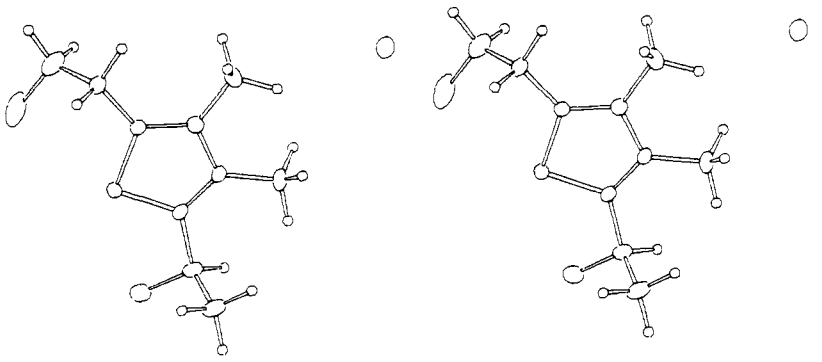
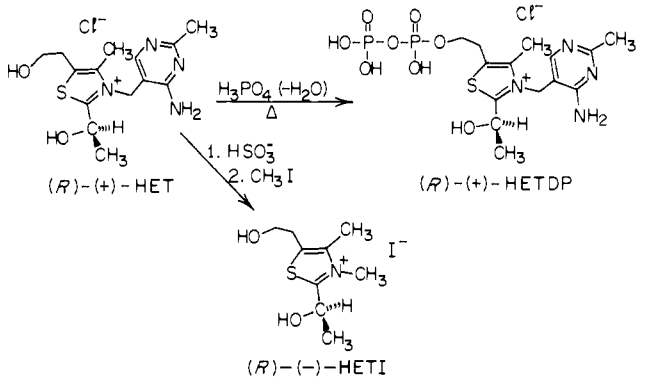


Figure 1. Stereoview of the structure of (+)-HETI. The individual structures are perspective drawings which refer to HETI in Scheme I.

Scheme I



followed by reaction of the thiazole with methyl iodide (Scheme I). (−)-HETI is crystalline [mp 123 °C, $[\alpha]^{25}_D$ −0.4 ± 0.1°; CD θ(300) 0, θ(262)−3200, θ(223) 0]. The material was used for crystallographic analysis (neither the dibenzoyltartrate salt of HET nor HET chloride provided suitable crystals). (−)-HET was carried through the same sequence and gave (+)-HETI ($[\alpha]^{25}_D$ +0.4 ± 0.1°). The structure and absolute stereochemistry of HETI were determined by low-temperature (130 K) single-crystal techniques by using a Nicolet P3 four-circle diffractometer equipped with a molybdenum tube and an over-the-tube liquid nitrogen cooling device. Cell constants were determined from 24 automatically centered reflections. The crystals are orthorhombic, space group $P2_12_12_1$ with a = 10.922 (3) Å, b = 16.426 (5) Å, c = 6.971 (2) Å at 130 K. Three sets of data, each including two complete subsets of Friedel mates, were measured with niobium-filtered Kα radiation to a resolution of sin θ/λ = 0.70 Å^{-1}. Set 1 included forms {±h, +k, +l}, set 2 {+h, ±k, +l}, and set 3 {−h, ±k, +l}. The data were recorded at 8.4° 2θ/min and at a tube setting of 60 kV, 15 mA. The data were corrected for Lorentz and polarization effects. A Gaussian numerical absorption correction was applied (μ_{Mo} = 2.67 mm^{-1}), and a time-dependent scale factor was determined and applied. There was a 3% decrease in the intensities of five standard reflections over the 13 h needed to record 12711 reflections in three sets. Statistical corrections and merging of data led to 2117 means with a weighted R_{sym} = 0.018. A preliminary structure of HETI was determined from room-temperature data using copper radiation; those structural parameters were directly refined against molybdenum data. Contributions from the anomalous scatters (S, I$^-$) were included in the structural refinement.[10] The positional and anisotropic thermal parameters of all 14 non-hydrogen atoms were refined. For the R enantiomer, the final goodness of fit and unweighted and weighted residuals are S = 1.89, R = 0.025, and R_w = 0.033. For the S enantiomer, the corresponding parameters are 2.49, 0.035, and 0.043, respectively. This unequivocally establishes that

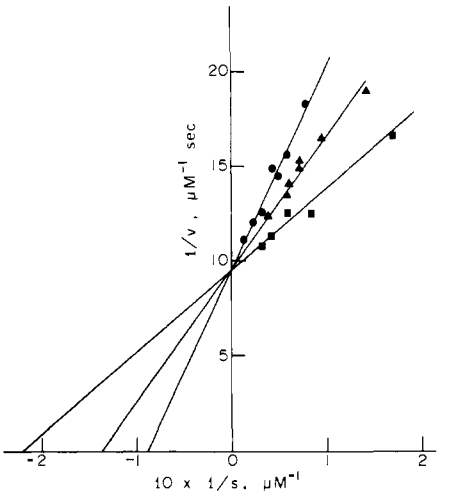


Figure 2. Activation of the apoeznyme of wheat germ pyruvate decarboxylase by (S)-(−)-HETDP (●), K_m = 12 μM; (R)-(+)-HETDP (▲), K_m = 7.3 μM; and TDP (■), K_m = 4.5 μM. V_{max} is 1.1 × 10^{-7} M s^{-1}.

(−)-HETI is the R enantiomer and thus (+)-HET and (+)-HETDP[6] are also R.

The structure of (R)-(−)-HETI is shown in Figure 1. The S–C2–C2a–O2a torsion angle is −102°. The S–C2–C2a–C2b angle is +15°. As in the structure of 2-(1-hydroxyethyl)-3,4-dimethylthiazolium bromide,[11] the sulfur atom is flanked by oxygen atoms in relatively close contact (5b-HO···S, 2.99 Å; 2a-HO···S, 2.86 Å).[11]

(R)-(+)-HETDP and (S)-(−)-HETDP were prepared from (R)-(+)-HET and (S)-(−)-HET, respectively.[6] Each enantiomer was free of TDP (<1%) by ^{1}H NMR and chromatographic analysis. The apoenzyme of wheat germ pyruvate decarboxylase[12] was incubated separately with (R)-(+)-HETDP, (S)-(−)-HETDP, and TDP. Each sample also contained NADH (3.3 μM), yeast alcohol dehydrogenase (0.20 mg), and $MgSO_4$ (1.25 mM) and was incubated for 8 min at 30 °C.[13] Reaction was initiated by the addition of pyruvate (30 mM) and observed at 340 nm. The results are presented in Figure 2.

Both enantiomers of HETDP fully activate the apoenzyme and thus the enzyme converts *both* enantiomers to enzyme-bound TDP and acetaldehyde, promoting the elimination by removal of the hydroxyl proton.[3,14,15] Since the K_m values reflect the rate of association of the apoenzyme with the coenzyme,[13,16] we conclude that the R enantiomer binds at a rate which is about 1.5 times that of the S enantiomer. This low stereoselectivity does not imply that the enzyme *produces* racemic HETDP or that the rates of conversion of the enzyme-bound species are similar. Certainly,

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the formation of the pyruvate adduct of TDP and the substitution process in which a proton replaces CO_2 to generate HETDP should be stereospecific[17] as is the case for the E1 subunit of pyruvate dehydrogenase.[5,14] The stereochemistry of these processes in enzymic and nonenzymic systems is the subject of ongoing studies.

Acknowledgment. Supported by an operating grant from NSERC Canada (R.K.), a NSERC scholarship (G.G.), and NIH Grant AM-19856 (G.T.D.).

Supplementary Material Available: Listing of crystal data and tables of final atomic positional and thermal parameters for HETI (1 page). Ordering information is given on any current masthead page.

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Streptonigrin Biosynthesis. 8. Evidence for the Involvement of a New Shikimate Pathway Product and a New Route to Quinolines[1]

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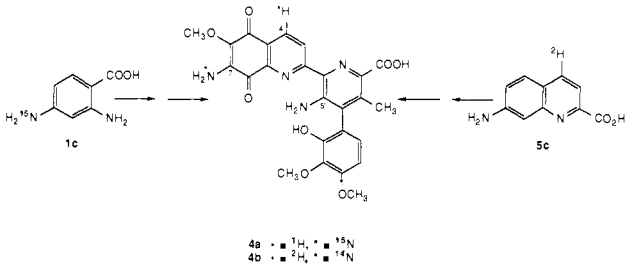
We have previously reported data[3,4] suggesting that a 4-aminoanthranilic acid (**1**), D-erythrose-4-phosphate (**2**), and β-methyltryptophan[5] (**3**) are the key precursors in biosynthesis of the anticancer antibiotic streptonigrin (**4**). As shown in Scheme I, these can be combined in sequences that lead either to a 7-aminoquinoline-2-carboxylic acid **5** (pathway A) or to a β-carboline **6** (pathway B) as the pivotal intermediate. We now report that pathway A is operative with 4-aminoanthranilic acid (**1a**) and 7-aminoquinoline-2-carboxylic acid (**5a**) as intermediates and that all three A-ring oxygenations occur at a later stage.

A fermentation in the presence of $^{18}O_2$ gas had yielded streptonigrin, labeled—among other positions—at C-5 and C-6 but not at C-8, suggesting that the C-8 oxygen was retained from a prearomatic precursor and that the hydroxylated compounds **1b** and **5b** were likely intermediates.[6] However, we recognized that because C-8 is the carbonyl of a vinylogous ester, an oxygen atom may have been introduced by a metabolic oxidation but subsequently lost by exchange,[7,8] either with the fermentation medium or during extractive workup. Indeed, when samples of authentic streptonigrin were stirred overnight in solutions of $THF/H_2{}^{18}O$ at pH 5.0 and at pH 10.5 and then reisolated and analyzed by ^{13}C NMR, it was found that ^{18}O had been incorporated to the extent of 15% and 30%, respectively, exclusively at C-8. Thus, neither the exact origin of the C-8 oxygen nor the oxidation level of the putative aromatic precursor(s) were certain at this point.

[4-^{15}N]4-Aminoanthranilic acid (**1c**) was then synthesized in three steps (22% overall yield)[9] utilizing $H^{15}NO_3$ (99% enriched).[10] A sample of the sodium salt of **1c** in 0.05 M pH 8.5 phosphate buffer was added under sterile conditions to shaken fermentations[11] of *Streptomyces flocculus*. Pulse feedings of 61.1 mg/15 mL, 20.8 mg/10 mL, 20.4 mg/10 mL, and 20.2 mg/10 mL were divided among the flasks (3 × 500 mL) at 24, 36, 48, and 60 h, respectively. At the termination of the fermentation, standard workup afforded 27.6 mg of pure **4a**. The ^{15}N NMR spectrum of **4a**[12] exhibited a single resonance at 73.6 ppm[13] that is attributable to the C-7 amine nitrogen of **4**.[5] Although the specific enrichment could not be calculated because of NOE due to proton decoupling, no resonance was detectable for the unenriched C-5′ amine nitrogen.[14]

[4-^{2}H]7-Aminoquinoline-2-carboxylic acid (**5c**) was next synthesized from the quinoline **7**[15] as shown in Scheme II. Reductive removal of chloride from **8** with deuterium gas afforded the labeled ester 9, and mild hydrolysis gave the amino acid **5c** in 25% overall yield.

The sodium salt of **5c** was fed by dividing pulses of 47.0, 44.5, 38.2, and 42.4 mg, each in 15 mL of buffer, among three 500-mL cultures at 28, 38, 48, and 58 h after inoculation, respectively. Standard workup afforded 20.6 mg of pure **4b** which was analyzed by ^{2}H NMR.[16] A singlet at δ 8.23[17] was observed corresponding



to a deuterium label at C-4. By comparison with the natural abundance deuterium signal for solvent Me_2SO (also employed as internal chemical shift reference), incorporation was determined to be 1.4%.

On the basis of these data it appears that streptonigrin is biosynthesized via pathway A with R = H (Scheme I), and unless there is a metabolic grid, it is unlikely that **1b** is also an intermediate. The evidence suggests that compound **1a** represents a new metabolite of the shikimate pathway,[19] while the involvement of **5a** reveals a fundamentally new biosynthetic pathway to the quinoline ring system.[20] This may be viewed (Scheme III) as

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(12) Spectrum taken on 22.0 mg in 0.4 mL of Me_2SO-d_6 with a Bruker AM 400 spectrometer at 40.5 MHz (sweep width = 2778 Hz, data points = 4K zero filled to 8K, Hz/pt = 0.68, acquisition time = 0.737 s, pulse width = 38°, line broadening = 2.0 s, relaxation delay = 1.2 s, no. of scans = 22 305).
(13) Relative to external [^{15}N]aniline, 56.5 ppm, obtained from MSD Isotopes.
(14) In earlier work both the C-7 amine and C-5′ amine peaks were of equal intensity in a natural abundance ^{15}N NMR spectrum. See ref 5. The C-7 amine nitrogen was also observed in the enriched and natural abundance samples by using a refocused decoupled INEPT sequence. This gave a signal-to-noise ratio approximately 3 times greater than that of the standard experiment described in ref 12. The C-5 amine nitrogen was not observed in this case, presumably due to rapid proton exchange eliminating the possibility of efficient polarization transfer.
(15) Heindel, N. D.; Bechara, I. S.; Lemke, T. F.; Fish, V. B. *J. Org. Chem.* **1967**, *32*, 4155.
(16) Spectrum taken on 10.0 mg in 0.4 mL of Me_2SO with a Bruker AM 400 spectrometer at 61.4 MHz (sweep width = 639 Hz, data points = 2K zero filled to 8K, Hz/pt = 0.16, acquisition time = 1.604 s, pulse width = 45°, no. of scans = 33 993).
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