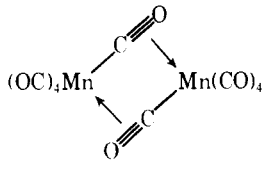
native formulations of $Mn_2(CO)_{10}^{\mp}$ can, of course, be postulated and I, for example, is by no means unlikely. Bridging



I

carbonyls of the type shown have been characterized crystallographically[12] and the structure is analogous to the well-known $(OC)_4Mn(\mu\text{-}Cl)_2Mn(CO)_4$.

Intensity-dependence studies of such reactions are the exact counterparts of the studies of the dependence of initial rates on initial concentrations of complex that have played a definitive role in establishing the existence of reversible fragmentation pathways in thermal reactions of metal-metal-bonded carbonyls.[13] Their essential role in the study of photochemical reactions of metal carbonyl clusters is evident.

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(2) The reversibility of equation 1 is indicated by the fact that limiting quantum yields for loss of the metal–metal-bonded carbonyls in such reactions are generally less than unity.[1,3] Participation of $Mn(CO)_4$ in the reaction scheme was not suggested[1a] and seems unlikely since none of the expected products, e.g., $Mn_2(CO)_8Cl_2$, was observed.

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(4) Runs were carried out using standard techniques. Solutions in 3-cm^3 cuvettes were rapidly stirred; 20–50% of the incident light was absorbed. Great care was necessary to purify the solvent and to remove oxygen by several thorough freeze–pump–thaw cycles. Solvent was used only when the quantum yield for decomposition of $Mn_2(CO)_{10}$ in the solvent was <0.02 mol $einstein^{-1}$. The decrease in absorbance due to $Mn_2(CO)_{10}$ at 343 nm was monitored up to ~10% reaction. Yields of $Mn(CO)_5Cl$ were measured by infrared spectrophotometry. The quantum yields quoted in Table I are effectively initial quantum yields. Within the accuracy of the measurements, the quantum yields for formation of $Mn(CO)_5Cl$ were twice those for loss of $Mn_2(CO)_{10}$ over the whole range of conditions used. No evidence was obtained for direct formation of any other product.

(5) This is probably the lowest $d\pi\sigma^*$ state.[1a,6]

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(7) Values of $\sigma(\phi_{obsd})$ are standard deviations of individual measurements of ϕ_{obsd} estimated so that 95% confidence limits can be obtained by doubling them.

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Stereochemical Analysis of the Homoserine Dehydrogenase Reaction and Preparation of Chiral 4-Deuteriohomoserines

Sir:

In the course of analyses of the stereochemical outcome of enzymic reactions at the γ carbon (C_4) of α-amino acids,[1] we have required a source of the physiological amino acid L-homoserine, stereospecifically deuterated at the prochiral C_4 alcoholic carbon. In this communication we report the preparation and the assignment of absolute stereochemistry to [4(*R*)- and [4(*S*)-^{2}H]-2(*S*)-homoserines from synthesis effected by homogeneous aspartokinase/homoserine dehydrogenase—a bifunctional enzyme from *E. coli*.[2] The useful activity for our purposes is the latter one, providing presumably chiral reduction of L-aspartate semialdehyde to L-homoserine at the expense of NADH oxidation. In turn this has required determination, reported here, of the previously unknown stereochemical outcome of this biosynthetic enzymic conversion of aldehyde to alcohol.

Two samples of [4-^{2}H]-L-homoserine were prepared enzymically. The first used [4(*S*)-^{2}H]-NADH[3] and L-aspartate semialdehyde with 5 units (5 μmol min^{-1} mg^{-1} of protein) of L-homoserine dehydrogenase[4] to yield 5.1 mg of pure [4-^{2}H]-L-homoserine (sample I) after LC purification (cation exchange, Whatman Partisil PXS 10/25 SCX). The second sample was obtained with unlabeled NADH and [4-^{2}H]-L-aspartate semialdehyde in a parallel incubation with analogous workup and a yield of 6.2 mg of [4-^{2}H]-L-homoserine (sample II). The [4-^{2}H]-L-aspartate semialdehyde was itself generated by ozonolysis of 4,5-dideuterio-L-allylglycine, in turn prepared by partial hydrogenation of L-propargylglycine with deuterium gas using Adams catalyst in ^2H_2O. The 4,5-dideuterio-L-allylglycine required rigorous purification from a small amount (5%) of unreacted acetylenic amino acid by LC, or else the ozonolysis products (presumably diketones) inhibit the homoserine dehydrogenase activity. The ozonolysis was carried out in 1 N $^2HCl/^2H_2O$ to yield, after enzymic aldehyde reduction, the anticipated [4-^{2}H]-L-homoserine (sample II).

That the two monodeuterio-L-homoserine samples did in fact have deuterium at the distinct (by virtue of isotopic substitution) diastereotopic methylene loci of carbon 4 was revealed by 270-MHz ^{1}H NMR as shown in Figure 1. The α and γ hydrogens have similar chemical shifts (α at 3.89 ppm and γ at 3.82 ppm). The spectrum of the γ hydrogens in nondeuterated L-homoserine appeared to be a triplet with secondary spliting into doublets by the α hydrogen. The coupling constants are 6.0 ($J_{\gamma\text{-}\beta}$) and 1.0 ($J_{\gamma\text{-}\alpha}$), respectively. The chemical shift of the γ hydrogens is 1031.4 Hz. The chemical shift of γ hydrogen of monodeuterio-L-homoserine (sample I) showed an upfield shift by 2.1 Hz, to 1029.3 Hz, from nondeuterated L-homoserine, and the other monodeuterio-L-homoserine (sample II) showed a further upfield shift to 1027.5 Hz. Line broadenings of the spectrum of γ hydrogens in sample I and sample II are due to deuterium coupling. Thus, *E. coli* homoserine dehydrogenase is stereospecific in reduction of the trigonal prochiral aldehyde group of L-aspartate semialdehyde. It remained then to determine absolute stereochemistry to assign upfield and downfield hydrogens in the NMR spectrum at γ (C_4) of the chiral [4-^{2}H]-2(*S*)-homoserine samples.

After some exploration we chose to degrade L-homoserine to 3-hydroxypropionate benzyl ester since it turned out to be an acceptable substrate for horse liver alcohol dehydrogenase, an enzyme known to remove only the *pro R* hydrogen from the oxidizable carbon of primary alcohols.[5] The degradation is shown in Scheme I. In the event we used a chiral [4-^{3}H]-L-homoserine sample **3** generated from homoserine dehydrogenase action on [4(*S*)-^{3}H]-NADH and L-aspartate semialdehyde, since we could then mix this species with [U-^{14}C]-

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Table I

expt	$^3H/^{14}C$ ratio in L-homoserine	$^3H/^{14}C$ ratio in 3-hydroxy-propionate benzyl ester[a]	total counts of ^3H, dpm	degree of completion,[b] %	^3H found in NADH	$^3H/^{14}C$ ratio in recovered 3-hydroxy-propionate benzyl ester **6**[c]
1	5.44	7.49	5.4×10^4	60	N.D.[d]	7.50
2	5.44	7.34	9.1×10^4	55	N.D.	7.29
3	5.46	7.25	5.5×10^4	58	N.D.	7.31
4	5.46	7.21	1.5×10^4	65	N.D.	7.25

[a] Ratio increase due to loss of ^{14}C upon decarboxylation of α-keto acid **4**. [b] Reaction mixture contained 5 μmol of 3-hydroxypropionate benzyl ester, 6 μmol of NAD in 1 mL of glycine buffer at pH 9.5. Reaction was started by addition of 2 mg of ADH and monitored by NADH absorbance at 340 nm. NADH was then purified by a DEAE anion-exchange column. Degree of completion was calculated by the optical density of product NADH. [c] Recovered by addition of cold 3-hydroxypropionate benzyl ester and purified repeatedly by thin layer chromatography (silica PF 254, $CHCl_3$). [d] N.D. = not detectable.

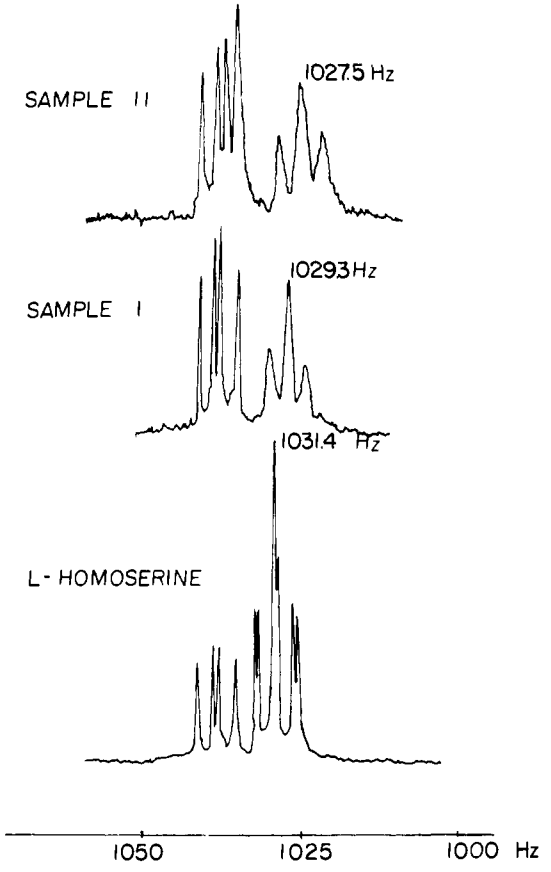
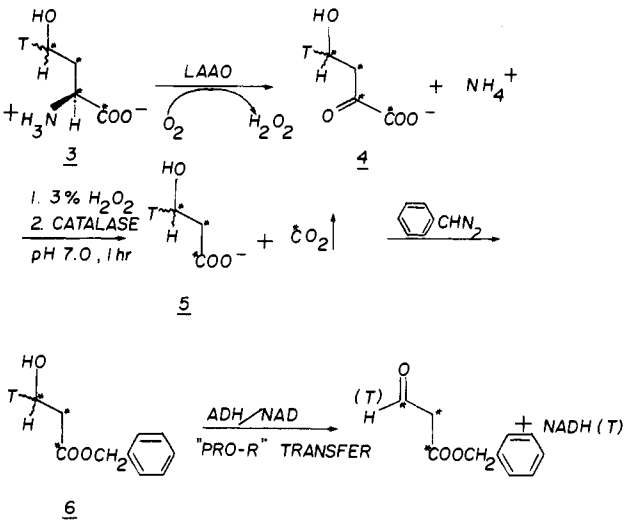


Figure 1.

L-homoserine (NEN) and follow $^3H/^{14}C$ ratios through the degradation scheme. Thus, 5.0 mg of L-homoserine, $^3H/^{14}C$ ratio 5.44, was completely oxidized by snake venom L-amino acid oxidase in 120 min at pH 7.4 and then the α-keto acid **4** was quantitatively decarboxylated by H_2O_2 as indicated. Since 3-hydroxypropionate **5** itself turns out not to be an alcohol dehydrogenase substrate, we extracted the acid into ethyl ether to remove phosphate salts and esterified with freshly distilled phenyldiazomethane. The benzyl ester **6** was then purified by TLC and subjected to alcohol dehydrogenase action. NADH formation was monitored at 340 nm for quantitative assay of alcohol oxidation and then separated from unreacted substrate and aldehyde product. Both NADH and unreacted 3-hydroxypropionate benzyl ester **6** were purified by chromatography and counted for radioactivity. Table I indicates the results of these experiments and reveals no transfer of tritium to NADH in four separate experiments showing that the tritium atom in the 3-hydroxypropionyl benzyl ester, and so at C_4 of the starting tritiated L-homoserine, is at the *pro S* locus.

Scheme I



Since no tritium was found in NADH, one might argue alternatively that tritium is in the transferable position and that there is a large tritium isotope effect upon breaking the C-H bond at the C_3 alcoholic carbon; then when 60% of the alcohol is oxidized, there might be very little tritium transferred to NADH (since <5% transfer could be detected, k_H/k_T would have to be ≥12). To eliminate this possibility, unreacted 3-hydroxypropionyl benzyl ester was reisolated from the incubations and purified, and the $^3H/^{14}C$ ratio checked. A k_H/k_T of ≥12 means that, at 60% reaction, a greater than twofold increase in the $^3H/^{14}C$ ratio would be observed. It was found that the $^3H/^{14}C$ ratio in the unreacted 3-hydroxypropionyl benzyl ester remains unchanged; tritium is indeed in the nontransferable *S* position of the alcoholic group. Thus, L-homoserine dehydrogenase transfers the *pro S* hydrogen from C_4 of NADH to the *si* face of bound L-aspartate semialdehyde, ending up at the *pro S* locus in C_4 of homoserine. Thus allows assignment of sample I of the [4-^{2}H]-L-homoserine (downfield C_4 H) as [4(*S*)-^{2}H]-L-homoserine and sample II (upfield C_4 H) as [4(*R*)-^{2}H]-L-homoserine (Figure 2). These deuterated samples will be useful both as reference standards to compare with 4-deuteriohomoserines generated in other enzymic

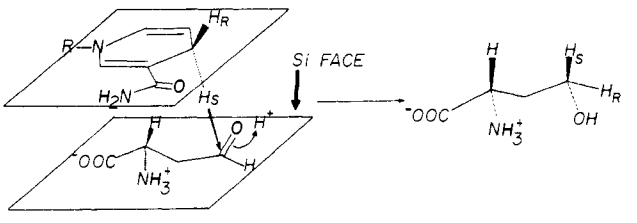


Figure 2.

transformations and also as chiral substrate samples to investigate stereochemical specificity of such γ-carbon processing enzymes as cystathionine γ-synthetase[6] and threonine synthase.[7] Finally, we have determined for the first time the stereochemical outcome of catalytic action of homoserine dehydrogenase, a central enzyme in biosynthesis of several (e.g., methionine, threonine) of the common α-amino acids.

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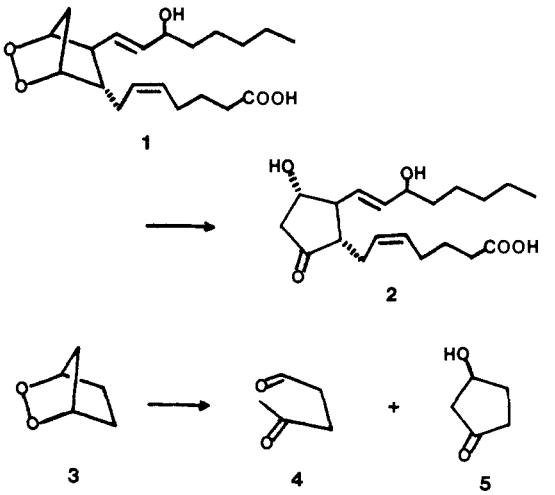
Prostaglandin Endoperoxides. 11. Mechanism of Amine-Catalyzed Fragmentation of 2,3-Dioxabicyclo[2.2.1]heptane[1]

Sir:

Disproportionation of prostaglandin endoperoxides (e.g., **1** → **2**) is a key step in the biosynthesis of D and E prostaglandins.[2] The fragmentation of 2,3-dioxabicyclo[2.2.1]heptane (**3**)[3] to levulinaldehyde (**4**)[4] which invariably accompanies disproportionation to 3-hydroxycyclopentanone (**5**) is fascinating since natural derivatives of **4** from **1** remain unknown. This paradox inspired us to examine carefully the mechanism of amine-catalyzed decomposition of **3**. We now report that amine catalysis of fragmentation (**3** → **4**) and disproportionation (**3** → **5**) are closely related mechanistically. Rate-determining cleavage of a bridgehead C-H bond generates a keto alkoxide which partitions between retro-aldol cleavage leading to **4** and protonation giving **5**.



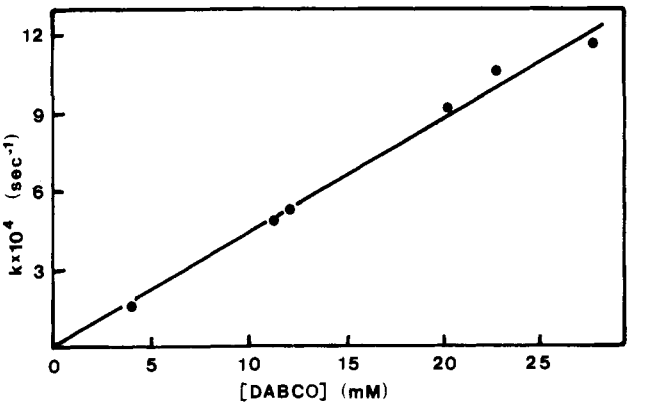


Figure 1. Correlation of pseudo-first-order rate constant for appearance of **4** with concentration of 1,4-diazabicyclo[2.2.2]octane.

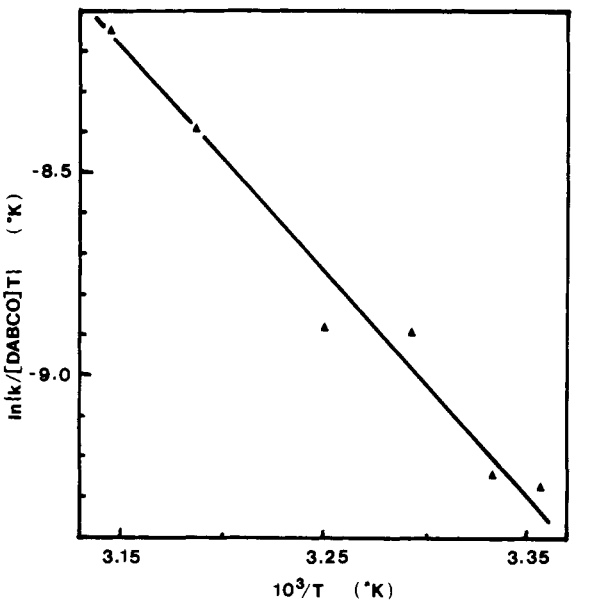


Figure 2. Temperature dependence of the pseudo-first-order rate constant (k) for appearance of **4**.

Decomposition of **3** in benzene solution in the presence of catalytic amounts of 1,4-diazabicyclo[2.2.2]octane (Dabco) was monitored by ^1H FT NMR. At 30.0 °C **4** and **5** are formed in 77-78 and 22-23% yields, respectively, over a range of catalyst concentrations from 4 to 28 mM. Over this range, the pseudo-first-order rate of appearance[5] of **4** is linearly correlated with catalyst concentration (Figure 1).

Rate constants were determined at various temperatures between 24.8 and 45.0 °C with 0.010 M Dabco and a 1.0 M initial concentration of **3**. These data show an excellent linear correlation of ln $(k/[\text{Dabco}]T)$ with $1/T$ where k is the observed pseudo-first-order rate constant for appearance of **4** (Figure 2). Activation parameters calculated from the rate constants listed in Table I are $\Delta H^{\ddagger} = 10.6 \pm 0.9$ kcal mol^{-1} and $\Delta S^{\ddagger} = -30 \pm 3$ eu.

Unimolecular thermal decomposition of **3** in nonpolar solvents, which gives 4,5-epoxypentanal almost exclusively, shows a considerably higher $\Delta H^{\ddagger} = 20.7 \pm 1.8$ kcal mol^{-1}.[1c] The large negative entropy of activation observed for the catalyzed fragmentation is consistent with a highly organized bimolecular transition state involving endoperoxide **3** and a molecule of catalyst.

Three different mechanistic types known for amine catalysis

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