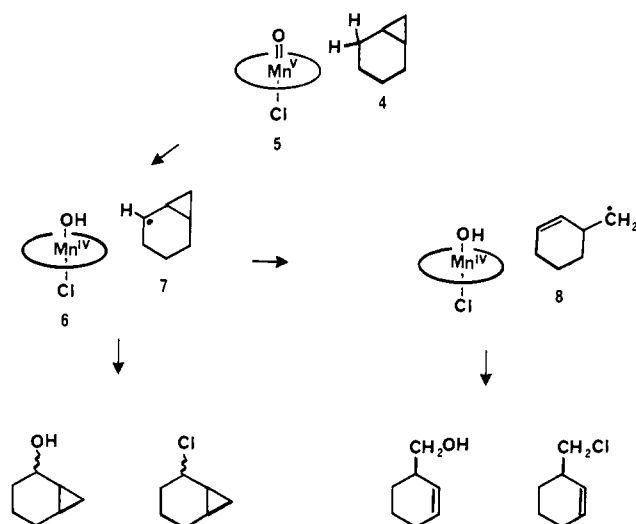


Scheme II



trast, cationic processes such as solvolyses or lead tetraacetate oxidation give cycloheptenyl rearrangement products.^{9b} The products of the oxidation of norcarane with iodosylbenzene catalyzed by $\text{TPPMn}^{\text{III}}\text{Cl}$ are shown in Scheme I.

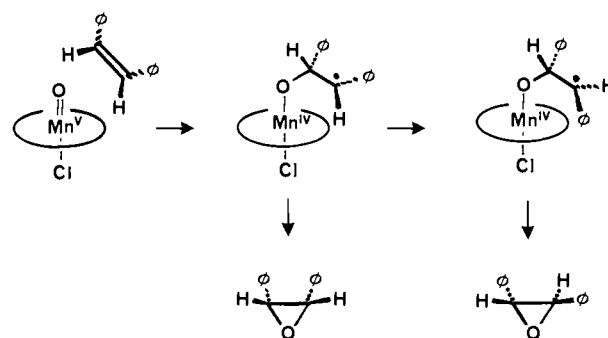
It is apparent that both oxygenated and chlorinated products characteristic of a free-radical reaction are obtained. A mechanism consistent with these observations is presented in Scheme II.

Hydrogen abstraction by the intermediate oxomanganese(V) complex **5** would give a chlorohydroxymanganese(IV) species (**6**) and a norcaranyl free radical (**7**). Ligand transfer from **6** to **7** could occur by homolytic displacement on the ligand or via a transient alkyl manganese species.¹⁰ In either case, the radical **7** must be long-lived enough to rearrange to the cyclohexenylmethyl radical **8** and to abstract halogen from the solvent. Aliphatic chlorination is also observed in benzene with $\text{TPPMn}^{\text{III}}\text{Cl}$ as the catalyst, showing that transfer of chlorine from the metal competes with oxygen transfer even when the solvent is not involved.¹¹

Another indication of the stepwise radical nature of the reaction of $\text{TPPMn}^{\text{III}}\text{Cl}/\text{PhIO}$ with hydrocarbons is apparent in the epoxidation of olefins. Oxidation of *trans*-stilbene under these conditions gave only *trans*-stilbene oxide in 53% yield. In contrast, *cis*-stilbene gave a 1.6:1 mixture of *trans*- and *cis*-epoxide, respectively, in 88% overall yield. Chloro(tetra-*o*-tolylporphinato)manganese(III) ($\text{TTPMn}^{\text{III}}\text{Cl}$) gave mostly *cis*-epoxide (2.8:1) in 87% yield. Thus, this epoxidation occurs with loss of stereochemistry at the double bond, and the distribution of products is sensitive to the substitution pattern on the porphyrin periphery. Addition of a manganese(V) intermediate to the carbon-carbon double bond to give a freely rotating free-radical intermediate is consistent with these observations (Scheme III).¹²

The lack of stereospecificity in olefin epoxidation and the relative abundance of evidence for free radicals in the reactions of alkanes with oxomanganese(V) complexes contrast with results we have obtained for the closely related iron and chromium porphyrins.^{2,3} We suggest that the triplet ground state expected for a high-spin $d^2 \text{Mn(V)=O}$ ion has affected the degree of concertedness of these reactions. The isolation and reactions of **1** suggest that the olefin oxygenation with $\text{O}_2/\text{NaBH}_4/\text{TPPMn}^{\text{III}}\text{Cl}$ recently reported by Tabushi¹³ may proceed through a similar oxomanganese(V) species. The role of such high ox-

Scheme III



idation state oxometalporphyrins in metalloenzyme function and the use of these catalysts in synthetic oxidation reactions are under continued study in our laboratories.

Acknowledgment. Support of this work by the National Institutes of Health (GM 25923) and the National Science Foundation (CHE-77-21849) is gratefully acknowledged. The National Science Foundation provided funds for the purchase of a GC-mass spectrometer.

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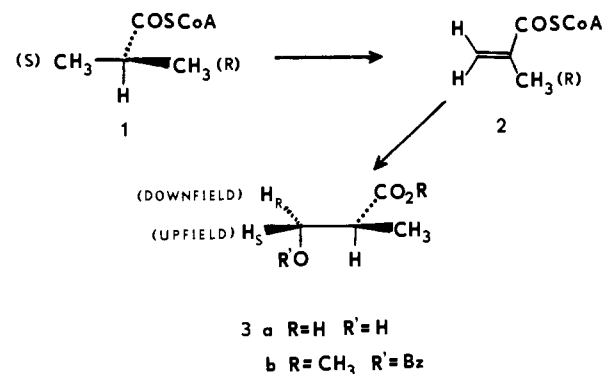
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Received April 7, 1980

Application of Tritium NMR Spectroscopy in the Determination of the Stereochemistry of Dehydrogenation of Isobutyryl CoA in *Pseudomonas putida*

Sir:

The principal catabolic pathway of L-valine proceeds via the metabolite isobutyryl CoA (**1**).¹ Dehydrogenation of **1** by a flavin-dependent² short-chain acyl CoA dehydrogenase³ produces



methacrylyl CoA (**2**). In rats⁴ and in *Pseudomonas putida* (ATCC 21244),⁵ hydrogens are eliminated from C-2 and from

(10) (a) For an excellent review, see: Kochi, J. K. *Free Radicals* 1973, 1, 591. (b) Organochromium intermediates have been suggested in the oxidation of olefins by chromyl chloride: Sharpless, K. B.; Teranishi, A. Y. Bäckvall, J. E. *J. Am. Chem. Soc.* 1977, 99, 3120.

(11) The yield of chloride is consistent with the amount of catalyst used. A more detailed account of ligand transfer oxidation will appear elsewhere.

(12) (a) The possibility of a four-membered oxametallacyclic intermediate should also be considered. (b) Kobayashi, Y. *Tetrahedron Lett.* 1972, 5093. (c) Ryang, H. S.; Foote, C. S. *J. Am. Chem. Soc.* 1980, 102, 2130.

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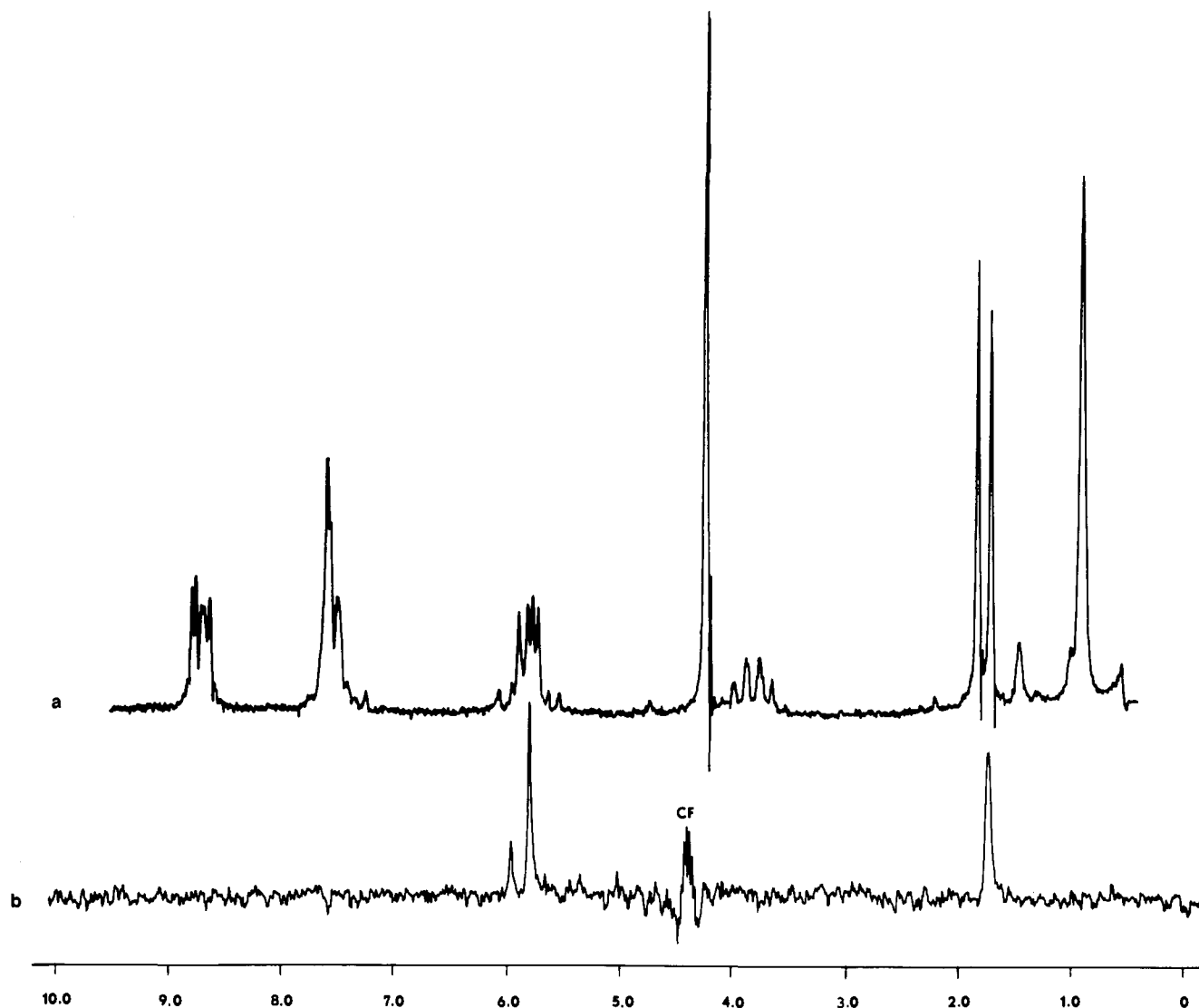


Figure 1. (a) 60-MHz ^1H NMR spectrum of **3b** (derived from **7a** + **7a**) + $\text{Eu}(\text{fod})_3$ in CDCl_3 (90%) + Me_4Si (10%). (b) 95.9-MHz ^1H -decoupled ^3H NMR spectrum of **3b** (from **7a** + **9a**) + $\text{Eu}(\text{fod})_3$. Chemical shifts are given in ppm ($\text{Me}_4\text{Si} = 0$).

the 2-*pro-S* methyl group in this transformation. We now report the results of an investigation involving the use of ^3H NMR spectroscopy which establishes that this dehydrogenation in *Ps. putida* proceeds with an antiperiplanar orientation of these eliminated hydrogens.

Our approach to this problem was to incubate chiral methyl-labeled isobutyrate with cells of *Ps. putida*, and to isolate the β -hydroxyisobutyric acid (β -HIBA) **3a** which accumulates in the medium.⁶ We recently showed that the hydration of methacrylyl CoA (followed by deacylation), **2** \rightarrow **3**, proceeds by syn addition of water to the *re, re* face of the double bond.⁷ Since the C-3 proton signals in the $\text{Eu}(\text{fod})_3$ -shifted ^1H NMR spectrum of the derivative **3b** were assigned in that work,⁷ the distribution of tritium at these positions resulting from chiral $\text{C}^1\text{H}^2\text{H}^3\text{H}$ methyl groups in isobutyrate could be evaluated by ^3H NMR.⁸

The required chiral methyl substrates for this study were prepared as shown in Scheme I. (*Z*)- and (*E*)-[3- $^2\text{H}_1$]methacrylic acids **4a** and **4b**⁹ were converted by Hassner's method⁹ to the corresponding benzyl esters **5a** and **5b**.¹⁰ Reduction of these with carrier-free tritium gas (5 Ci) in the presence of chlorotris(triphenylphosphine)rhodium(I) in benzene¹¹ gave mixtures consisting of equal amounts of the 2*S*,3*S* and 2*R*,3*R* diastereomers **6a** and **8a** (from **5a**), or the 2*S*,3*R* and 2*R*,3*S* diastereomers **6b** and **8b** (from **5b**), plus, presumably, substantial amounts of unreacted

(9) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475-4478.

(10) The syntheses of **4a** and **4b** by Zn/Hg reduction of 3-bromo-2-methylprop-2-enoic acid produce a substantial amount of isobutyric acid as a byproduct. This was removed by preparative GLC (5% Carbowax 20 M, 145 $^\circ\text{C}$) of the corresponding benzyl esters **5a** and **5b**. The stereospecificity of the labeling (ca. 95%, by ^1H NMR) was not affected by this purification.

(11) Reduction of unlabeled benzyl methacrylate under identical conditions but with a volume of $^2\text{H}_2$ approximately equal to that used for $^3\text{H}_2$ reductions gave a product consisting of benzyl isobutyrate (ca. 30%) plus unreacted starting material (ca. 70%). The GLC-purified benzyl isobutyrate from this reduction showed no α - ^1H signal in the ^1H NMR spectrum, and showed a singlet for the methyls. It is therefore assumed that the reduction occurs without hydrogen scrambling. In contrast, reduction with $^3\text{H}_2$ over Pd/C showed extensive hydrogen scrambling. It is further assumed that the $(\text{Ph}_3\text{P})_3\text{RhCl}$ -catalyzed $^3\text{H}_2$ reduction proceeds exclusively with *cis* addition. While we have no direct proof of this, the asymmetric distribution of tritium signals in the biosynthetic products supports stereospecific labeling in the precursors. It is unlikely that the hydrogenation would proceed by *trans* addition. For another claimed, but unproven, synthesis of a chiral methyl group by reduction of an α -acetoxycrylic ester, see: Fryzuk, M. D.; Bosnich, B. *J. Am. Chem. Soc.* **1979**, *101*, 3043-3049.

(6) Goodhue, C. T.; Schaeffer, J. R. *Biotechnol. Bioeng.* **1971**, *13*, 203-214.

(7) Aberhart, D. J.; Tann, C. H. *J. Chem. Soc., Perkin Trans. 1* **1979**, 939-942.

(8) For references on recent applications of ^3H NMR spectroscopy in biosynthetic investigations, see: (a) Altman, L. J.; Han, Y. C.; Bartolino, A.; Handy, G.; Laungani, D.; Muller, W.; Schwartz, S.; Shanker, D.; de Wolf, W. H.; Yang, F. *J. Am. Chem. Soc.* **1978**, *100*, 3235-3237. (b) Elvidge, J. A.; Jaiswal, D. K.; Jones, J. R.; Thomas, R. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1080-1083. (c) Chambers, V. M. A.; Evans, E. A.; Elvidge, J. A.; Jones, J. R. "Tritium Nuclear Magnetic Resonance Spectroscopy", The Radiochemical Center: Amersham, England, 1978. (d) Bloxidge, J. P.; Elvidge, J. A.; Jones, J. R.; Mane, R. B.; Saljoughian, M. *Org. Magn. Reson.* **1979**, *12*, 574-578.

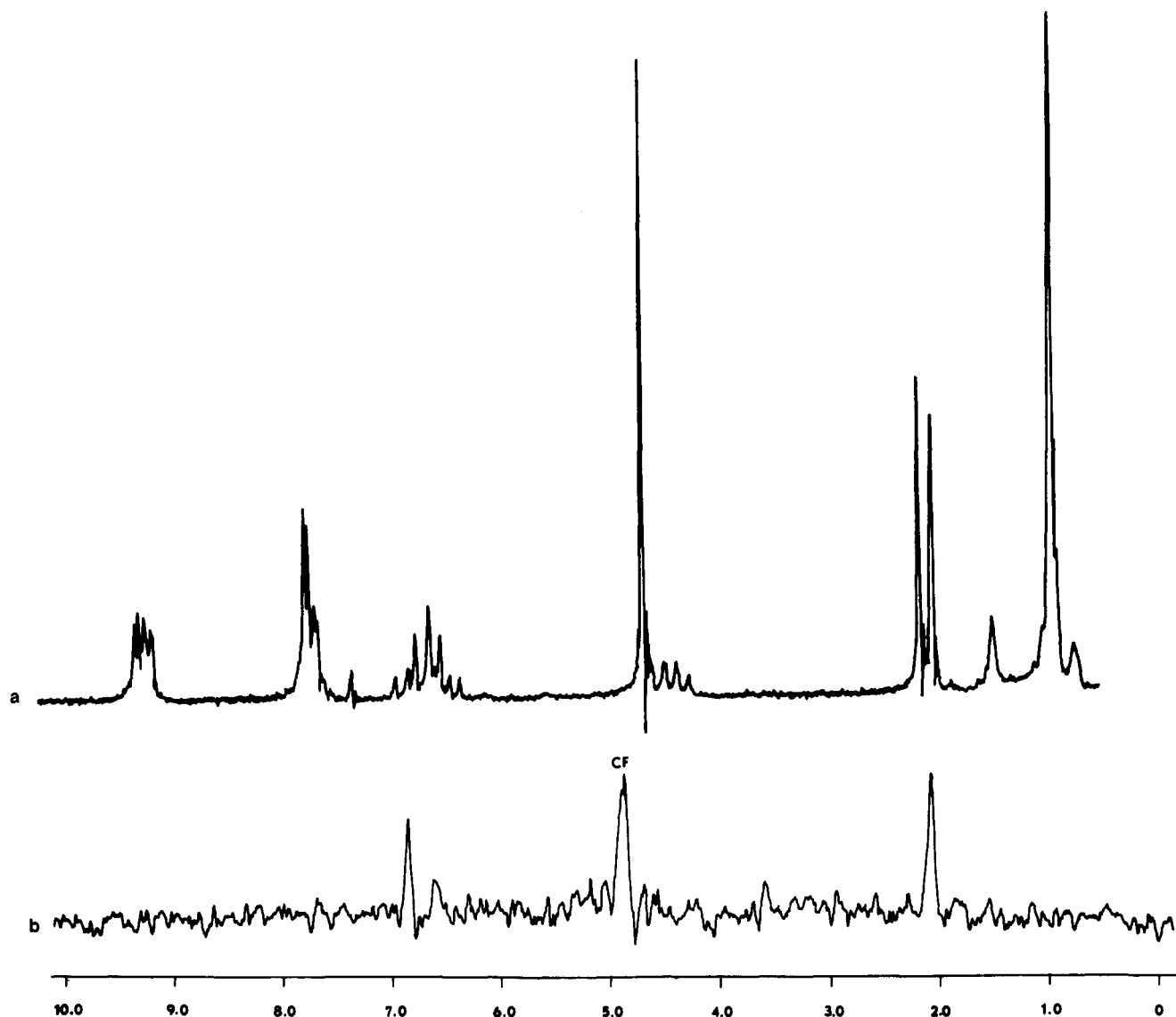


Figure 2. (a) 60-MHz ^1H NMR spectrum of **3b** (derived from **7b** + **9b**) + $\text{Eu}(\text{fod})_3$ in CDCl_3 (90%) + Me_4Si (10%). (b) 95.9-MHz ^1H -decoupled ^3H NMR spectrum of **3b** (from **7b** + **9b**) + $\text{Eu}(\text{fod})_3$. Chemical shifts are given in ppm ($\text{Me}_4\text{Si} = 0$).

5a or **5b**.¹² The benzyl esters were then saponified under mild conditions with excess KOH in EtOH ¹³ to yield the K^+ salts of **7a** and **9a** or **7b** and **9b**, respectively. These products (ca. 2 Ci each) were each then mixed with unlabeled potassium isobutyrate and adjusted to pH 7.4 with HCl .¹⁴ These mixtures were then incubated with cells of *Ps. putida* (ATCC 21244), pregrown in a medium containing 5 g/L isobutyrate as the sole source of carbon. The resultant β -HIBA **3a** was isolated and purified as the derivative **3b**. A total of ca. 20 mCi of **3b** was obtained from each of the two incubations. The product solutions were mixed with $\text{Eu}(\text{fod})_3$ to shift the C-3 CH_2 proton signals to ca. δ 5.5–7.0.¹⁵

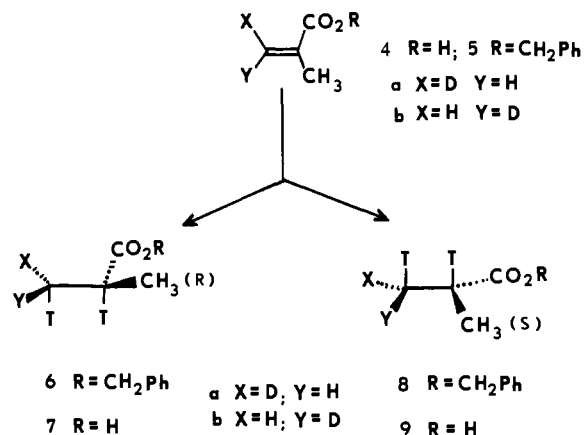
(12) Due to the high amount of radioactivity in these products and their slight volatility, analysis of the mixture was not carried out. The presence of a small amount of non-tritium-labeled methacrylate in the biosynthetic precursor in no way interferes with the biosynthetic experiment.

(13) Treatment of benzyl [$2\text{-}^3\text{H}_1$]isobutyrate with KOH/EtOH under identical conditions followed by reesterification⁹ of the isobutyrate with benzhydryl alcohol gave a product showing no exchange of the α -deuteron with the medium.

(14) The chiral methyl-tritiated isobutyrate (ca. 2 Ci, ca. 0.034 mmol) were mixed with 5.7 mmol of unlabeled potassium isobutyrate. Thus, ca. 0.6% of all isobutyrate molecules in the mixture contained tritium.

(15) The required amount of $\text{Eu}(\text{fod})_3$ was estimated by using a similar unlabeled sample of **3b** to avoid manipulation of unsealed ^3H samples in the NMR laboratory. No attempt was made to shift these signals to exactly the same position in both samples. The C-3 hydrogen signals of interest are well separated in the region of δ 5.5–7.0.

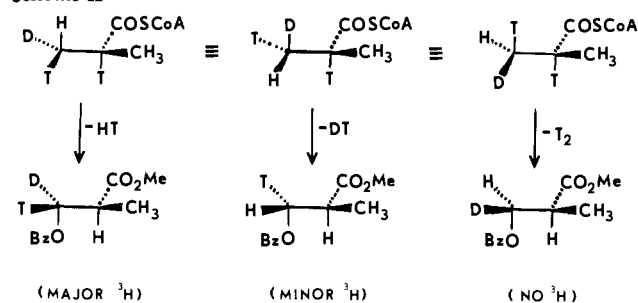
Scheme I^a



^a Synthetic pathway for chiral methyl isobutyrate.

The ^1H and ^3H NMR spectra were then recorded (Figures 1 and 2).¹⁶⁻¹⁸

(16) ^1H NMR spectra were recorded on a Varian EM-360 instrument.

Scheme II^a

^a Predicted ³H labeling patterns of metabolites from 7a for anti elimination of hydrogens from C-2 and C-3 of isobutyryl CoA (followed by syn hydration on *re, re* face of double bond, loss of CoA, and preparation of methyl ester benzoate).

Figure 1b shows the ¹H-decoupled ³H NMR spectrum of 3b obtained from the 7a + 9a isobutyrate mixture. Tritium signals at δ 5.80 (3-*pro-S* H) and 5.96 (3-*pro-R* H) result from the metabolism of the 2-*pro-S* chiral methyl of 7a whereas the signal at δ 1.74 is derived from the unoxidized 2-*pro-R* chiral methyl of 9a. The ³H chemical shifts correspond closely with the analogous proton signals in Figure 1a. The relative tritium signal intensities at δ 5.80 and 5.96 are consistent with expectations for an anti elimination of hydrogens from 7a, assuming $k_H > k_D > k_T$ for loss of the C-3 hydrogen atom (cf. Scheme II).¹⁹

Figure 2b shows the ¹H-decoupled ³H NMR spectrum of 3b derived from the 7b + 9b isobutyrate mixture. Although the ³H chemical shifts are not identical with those in Figure 1b [due to the presence of more Eu(fod)₃ in the solution], they correspond well with the relevant ¹H signals (Figure 2a). The intensity distribution of the two low-field ³H signals complements that observed in Figure 1b. The two spectra, Figures 1b and 2b, require the conclusion that the dehydrogenation of isobutyryl CoA in this organism proceeds by antiperiplanar elimination of hydrogens from C-2 and C-3.²⁰ The stereochemistry thus parallels that observed for the dehydrogenation of *n*-butyryl CoA by short-chain acyl CoA dehydrogenase from pork liver,²¹ and for the dehydrogenation of (2*S*)-2-methylbutyryl CoA in *Datura innoxia* plants.²²

Acknowledgment. We express our thanks to Frank Shea and Professor Alan A. Jones, Clark University, Worcester, Massa-

(17) ³H NMR spectra were recorded at 95.9 MHz on a Bruker SXP 22/100 spectrometer. Samples were dissolved in ca. 0.4 mL of a mixture of CDCl₃ (90%) and Me₄Si (10%) and were sealed under N₂ in standard thin-wall 5-mm tubes. Tritium chemical shifts were determined relative to Me₄Si by the "ghost referencing technique".^{8d} Spectra were run without NOE suppression to minimize spectrum acquisition time. The operation of NOE in the proton-decoupled spectra is expected to introduce only minor changes in relative signal intensities.¹⁸ No apparent change in the relative intensities observed in Figure 1b was observed in the ¹H-coupled ³H NMR spectrum of 3b derived from 7a + 9a (see ref 19). However, the signal-to-noise ratio was substantially lower in the ¹H-coupled spectrum. The pattern appearing at ca. δ 4.5-5.0 (designated CF, Figures 1, 2) is the center frequency of the instrument and not a tritium resonance.

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(19) The signal assignments are also supported, in part, by ¹H couplings observed in the proton-coupled ³H NMR spectrum of 3b derived from 7a + 9a. The δ 1.74 signal appeared as a doublet of doublets ($J_1 = 7.7$ Hz, $J_2 = 13.4$ Hz), due to vicinal coupling with the C-2 hydrogen and geminal coupling with the single ¹H on the chiral methyl group, respectively. The pattern shows that all residual methyl groups in the isolated 3a (and therefore also in the starting material) which bear tritium also contain one deuterium atom. The peak at δ 5.80 appeared as a doublet ($J = 6.6$ Hz), supporting attachment of this tritium atom to carbon also bearing deuterium. Unfortunately, the tritium signal at δ 5.96 was only poorly resolved from background noise. Couplings of ³H with ²H could not be resolved in either the ¹H-coupled or the decoupled spectra.

(20) The results also support the involvement of a substantial isotope effect in the loss of the C-3 hydrogen.

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chusetts, for the ³H NMR spectra. We also thank Dr. Eliahu Caspi for provision of hot lab facilities and for stimulating discussions. We thank Professor John A. Elvidge for provision of valuable information on tritium referencing procedures. The ³H NMR spectra were recorded on an instrument supported in part by National Science Foundation Equipment Grant CHE77-09059. This work was supported by Grant GM 24420 from the National Institute of General Medical Sciences.

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Received May 27, 1980

Angular Momentum, the Principle of Equal a Priori Probabilities, and "Intermolecular Entropy Corrections" in Equilibria of Ions and Molecules in the Gas Phase

Sir:

Two years ago, Lias and Ausloos¹ first suggested that the entropy term associated with gaseous equilibria of ions and neutral species may require a term to account for the effect of the long-range (r^{-4}) interaction between the two species even at pressures less than 1 torr. On the basis of a classical collision theory argument, they proposed¹⁻⁴ a pressure-independent correction term of a form deriving from collision frequencies (Z) in the forward and reverse directions (eq 1 and 2), where P designates $K_{eq} = (Z_f/Z_r)(P_f/P_r) \exp(-\Delta H/RT) =$

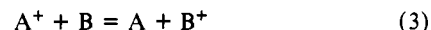
$$\exp(\Delta S/R - \Delta H/RT) \quad (1)$$

$$\Delta S = R \ln (Z_f/Z_r) + R \ln (P_f/P_r) = \Delta S_{int} + \Delta S_{stat} \quad (2)$$

the conventional "steric" factor. The first term on the right side of eq 2 is interpreted as an intermolecular entropy term (ΔS_{int}) and as a correction to the entropy (ΔS_{stat}) calculated on the basis of statistical mechanics. Various arguments against this proposal based on microscopic reversibility, transition-state theory,⁵ and statistical mechanics have been offered, and the views of the proposers on these have been discussed extensively.⁶ We present here an analysis employing a simple model system which demonstrates that at the low pressures (less than 1 torr) at which such equilibria are studied intermolecular correction terms based on the kinetic argument violate an elementary probability theorem and conservation of angular momentum; since such a term would have to apply to all systems, it cannot exist.

Thermodynamic expressions will contain a concentration-dependent intermolecular term, which may be derived by statistical considerations such as the well-known Debye-Hückel treatment, at densities four or five orders of magnitude greater than the highest pressures used in experimental studies of ionic equilibria in the gas phase; these terms are not a subject of this communication.

We choose for demonstration a charge-transfer reaction (eq 3) between atomic species A and B of exactly equal ionization potentials and degeneracies but different polarizabilities, α_A and



α_B . The elementary, fundamental principle that all quantum states of equal energy have equal a priori probabilities underlies equipartition and all statistical mechanics; it requires without mathematical or further conceptual development that the equilibrium constant for reaction 3 is unity, $K_{stat} = 1$, at all temperatures, as long as the densities are sufficiently low so that the intermolecular interactions cannot affect the ionization potentials of A and B

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