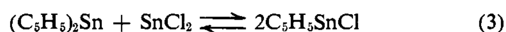
$C_5H_5$ ligand from delocalized to diene type; and (iv) change from centrally $\sigma$ bonding (pseudo-$\pi$) to localized $\sigma$ bonding of the rings.

Nmr spectra of tin(II)[1b] and tin(IV) cyclopentadienyls[6,16] have been recorded. The $C_5H_5$ protons in II are downfield of I. In cyclopentadienyliron derivatives the $Fe^{57}$ Mössbauer IS and nmr $\tau$ values are related linearly such that an increase in IS of 0.3 mm/sec is accompanied by an increase of nearly one $\tau$ unit, taking the extremes of the scale.[17] For tin an increase in IS of 2.7 mm/sec is accompanied by a *decrease* of a few hundreths of a $\tau$ unit. Recalling the difference in signs of the fractional change in the nuclear charge radii for iron and tin,[18] these observations are corroboratory. The large substituent-induced nmr shifts for iron ($\tau$ 3.92–5.87),[17] are not seen in the various organotin(IV) cyclopentadienyls ($\tau$ 4.05–4.13),[6] nor in the small shift (barely a few cycles at 60 MHz) accompanying the change in tin valence state.

The single sharp resonances of the $C_5H_5$ protons have been interpreted in terms of stereochemically nonrigid cyclopentadienyltin(IV) systems, and temperature-variable spectra have been reported for several compounds.[16b,19] The observation of spin–spin coupling shows that the processes occurring are *intra*molecular. The nmr spectrum of I[1b] [$J(Sn^{117,119}–C–H^1)$ = 15.9 Hz] is unchanged at $-90°$, where crystallization takes place from most suitable solvents, but the tin satellites disappear at 125° in xylene (the $C_5H_5$ signal remains sharp), denoting the onset of dissociative processes. Other *inter*molecular processes are brought about by exchange with tin(II) chloride and trimethyltin(IV) chloride.



$$(C_5H_5)_2Sn + SnCl_2 \rightleftharpoons 2C_5H_5SnCl \quad (3)$$

$$(C_5H_5)_2Sn + (CH_3)_3SnCl \rightleftharpoons C_5H_5SnCl + (CH_3)_3SnC_5H_5 \quad (4)$$

No satellites are observed on the sharp $C_5H_5$ singlets, even at $-80°$. The position of the equilibrium in (4) can be established from the methyltin proton resonance relative to the two pure methyltin species.

Information concerning the conformational integrity of I with phase comes from ir and Mössbauer spectra in solution, which are very similar to those for the solid; the ir can be used to rule out a diene-type cyclopentadienyl ring in either phase.[20] No peaks above the parent ion are seen in the mass spectrum, where the most prominent peak is the monotin fragment at 184 ($C_5H_5Sn^+$ = 100%) followed by 119 ($Sn^+$ = 13%). This behavior differs from that of the $\sigma$-cyclopentadienyls such as $C_5H_5CuP(C_2H_5)_3$ which does not show the $C_5H_5Cu^+$ peak[21] and from that of ferrocene and other $\pi$-bonded species where prominent parent ions are seen (*vs.* 249 [$(C_5H_5)_2Sn^+$ = 4.6%]) but resembles the vanadium and nickel examples.[22] Like both ferrocene and nickelocene, I gives rise to fragments at 158 ($SnC_3H_3^+$ = 3.7%) and 144 ($SnC_2H^+$ = 3.0%). The former, presumably a cyclopropenium tin ion, has been previously observed with cyclopentadienyl–transition metal compounds.[22]

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**P. G. Harrison, J. J. Zuckerman**
*Department of Chemistry*
*State University of New York at Albany*
*Albany, New York 12203*

*Received July 17, 1969*

## The Carboxyl Carbon Isotope Effect on the Enzymatic Decarboxylation of Glutamic Acid

*Sir:*

Heavy-atom isotope effects have been used to study the mechanisms of a number of chemical reactions,[1–4] but little use has been made of heavy-atom isotope effects in biochemical systems. Seltzer, Hamilton, and Westheimer reported only a very small carbon isotope effect on the enzymatic decarboxylation of oxalacetic acid.[5] Other studies of heavy-atom isotope effects have provided little useful information about mechanism because of unexplained variations of isotope effect from experiment to experiment.[6–8] The extreme sensitivity of isotope effects to contamination requires that such measurements be made and reported with great attention to experimental technique.

We have measured the carboxyl carbon-13 isotope effect on the decarboxylation of glutamic acid catalyzed by an enzyme from *E. coli.*[9] The isotope effect is $k^{12}/k^{13}$ = 1.0172 at 37°, pH 4.7. Isotope effects of this type can provide much useful information about the correctness of the Michaelis–Menten mechanism and about the nature of the decarboxylation transition state.

For each experiment freshly prepared solutions of 0.01 *M* L-glutamic acid in 0.1 *M* pyridine hydrochloride buffer, pH 4.7, were freed of $CO_2$ by bubbling $CO_2$-free nitrogen through the solutions for 30 min. The air in each reaction flask was displaced with nitrogen before

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Table I. Carboxyl Carbon-13 Isotope Effects on the Enzymatic Decarboxylation of Glutamic Acid at pH 4.7, 37°

| Enzyme spec act.[a] | Isotope ratios[b] 5% reacn | 100% reacn | $k^{12}/k^{13}$ |
|---|---|---|---|
| 17 | 0.014062 | 0.014287 | 1.0170 |
| 17 | 0.014070[c] | 0.014303[d] | 1.0176 |
| 17 | 0.014071 | 0.014303[d] | 1.0175 |
| 140 | 0.014079 | 0.014304 | 1.0169 |
| 140 | 0.014083[e] | 0.014309 | 1.0170 |
| 140 | 0.014084 | 0.014305 | 1.0166 |
| 140 | 0.014067[c] | 0.014307 | 1.0181 |
| | | Mean | 1.0172 ± 0.0004 |

[a] Micromoles of glutamic acid decarboxylated per milligram of enzyme per minute in 3 ml of 0.025 $M$ glutamic acid, pH 4.9, 37°. [b] $M/e$ 45/44, corrected to tank standard 0.014150. [c] Carried to only 2.5% reaction. [d] Same sample. [e] $10^{-5}$ $M$ pyridoxal 5'-phosphate added.

the flask was closed. The enzyme used in these studies (prepared as described previously[9]) was freed of low molecular weight contaminants before use by chromatography on a short column of Sephadex G-25 using freshly degassed buffer. Each flask containing the 0.01 $M$ glutamic acid was equilibrated at 37.0° and an appropriate amount of enzyme was added through a serum cap. After a suitable length of time the reaction was stopped by the addition of concentrated $H_2SO_4$. The evolved carbon dioxide was freed of water, nitrogen, and other contaminants by standard procedures and its isotopic composition was measured on a Nuclide RMS 6-60 isotope-ratio mass spectrometer. All measurements were made relative to a standard $CO_2$ sample. The isotope effect was calculated by comparison of the isotopic composition of a sample of $CO_2$ obtained after 5% reaction with one obtained after complete decarboxylation. The isotope effect is then given by eq 1,[1]

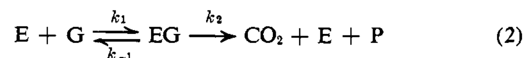
$$k^{12}/k^{13} = (R_{100} - R_{17})/(R_5 - R_{17}) \quad (1)$$

where $k^{12}$ and $k^{13}$ are the rate constants for the $^{12}C$ and $^{13}C$ species, respectively, $R_5$ and $R_{100}$ are the measured isotope ratios ($m/e$ 45/44) for the samples obtained at 5 and 100% conversion, respectively, and $R_{17}$ is the isotope ratio for carbon dioxide containing $^{17}O$, $^{12}C^{17}O^{16}O/^{12}C^{16}O_2$. The value 0.0008 has been used for $R_{17}$, and the calculated isotope effect is quite insensitive to the value of this ratio.

The correctness of the results was indicated by several tests: the isotope effect was constant from experiment to experiment; precisely the same isotope effect was obtained with different enzyme preparations; added $10^{-5}$ $M$ pyridoxal 5'-phosphate had no effect on the isotope effect; the absolute isotope abundances were constant from experiment to experiment; exactly the same isotope effect was obtained when a sample was allowed to proceed to only 2.5% reaction instead of 5% reaction.

The results of seven determinations of the isotope effect are summarized in Table I. The isotope ratios given in this table are not actual isotopic abundances, but are instead uncorrected decade settings on the isotope-ratio mass spectrometer. Thus a standard $CO_2$ sample gives a ratio $m/e$ 45/44 of 0.014150. The decade settings are directly proportional to isotopic abundances and can be used directly in eq 1. The reported ratios are estimated to be correct to ±0.000002. The mean of seven determinations is 1.0172 ± 0.0004. The pH of these measurements, 4.7, is within the range of maximum activity of the enzyme.[10]

The isotope effect can be related to the rate constants of the classical Michaelis–Menten formulation, eq 2, where E represents enzyme, G is glutamic acid, and P



$$E + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EG \xrightarrow{k_2} CO_2 + E + P \quad (2)$$

is product. The first step in eq 2 is the formation of an enzyme-bound Schiff base between glutamic acid and pyridoxal 5'-phosphate. Since there is no change in bonding to the isotopic atom in this step, there will be no carbon isotope effect on $k_1$ or $k_{-1}$, and the observed isotope effect is given by eq 3.[5] Thus the observed

$$\frac{k^{12}}{k^{13}} = \frac{k_2^{12}(k_{-1} + k_2^{13})}{k_2^{13}(k_{-1} + k_2^{12})} \quad (3)$$

isotope effect is equal to the isotope effect on $k_2$ reduced by a factor related to the extent to which $k_2$ is rate determining.[11] The presence of a sizable carbon isotope effect indicates that $k_2$ is not fast compared with $k_{-1}$. However, the mere occurrence of an isotope effect does not indicate that $k_2$ is rate limiting.

We cannot be sure without further consideration whether the isotope effect which we have observed is the one which would be expected if $k_2$ is much smaller than $k_{-1}$. It is instructive to compare this isotope effect with those obtained in nonenzymatic decarboxylations,[13] which are usually in the range 1.02 to 1.04. However, we do not necessarily expect the isotope effect on $k_2$ in eq 2 to be as large as those observed in nonenzymatic systems because of the greater transition-state stabilization provided by pyridoxal 5'-phosphate and the consequent smaller amount of carbon–carbon bond breaking expected at the transition state. Thus we can conservatively estimate that in this case $k_2$ is smaller than $k_{-1}$ and decarboxylation is the rate-limiting step. Whether these two rate constants differ by a small amount or by an order of magnitude or more is the subject of continuing investigation.

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(11) It should be noted that Jencks' interpretation of the isotope effect on the decarboxylation of oxalacetate[5,12] is incomplete. The rate-limiting step in the overall reaction may not be the same as the rate-limiting step in the decarboxylation process, and a carbon isotope effect could be observed even if decomposition of the enzyme–product complex were rate determining.

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Marion H. O'Leary
*Department of Chemistry, University of Wisconsin*
*Madison, Wisconsin 53706*
*Received July 3, 1969*

## A Novel Carbonium Ion Rearrangement in the *exo*-Tricyclo[4.2.1.0$^{2,5}$]non-3-ene Series

*Sir:*

Considerable interest has been focused recently on the extensive participation (*ca.* $10^{14}$ rate acceleration factor)