$$\text{observed}\ \frac{k^{12}}{k^{13}} = \frac{(k_5{}^{12}/k_5{}^{13}) + (k_5/k_4)}{1 + (k_5/k_4)}$$

By analogy with model studies[11] the isotope effect on the decarboxylation step, $k_5{}^{12}/k_5{}^{13}$, is likely to be in the range of 1.04–1.06. Because of the similarity of the electronic structures of all substrates examined in this study, it is likely that this value will be similar or identical for all substrates. The variations in observed isotope effects are thus expected to appear primarily in the ratio k_5/k_4, which reflects the difference in free energy between the transition state for the Schiff base interchange step and that for the decarboxylation. Qualitatively, a carbon isotope effect near unity will be observed if Schiff base interchange is rate determining, whereas an isotope effect in the range of 1.04–1.06 will be observed if decarboxylation is rate determining.

The carbon isotope effects observed with the rapidly reacting substrates arginine and canavanine indicate that in these cases neither decarboxylation nor Schiff base interchange is entirely rate determining. This observation continues a pattern seen with other decarboxylases, whereby for the natural substrate no single step is entirely rate limiting.[12]

The carbon isotope effects observed with the slowly reacting substrates homoarginine and norarginine are in the range expected for rate-determining decarboxylation. Thus, the low rates of decarboxylation of these substrates reflect low rates of the decarboxylation step, and the specificity of arginine decarboxylase toward these substrates is primarily manifested in the decarboxylation step.[13]

Why is specificity manifested principally in the decarboxylation step? All substrates examined have the amino, carboxyl, and guanidino groups required for substrate binding. The lack of a correlation between Michaelis constant and substrate length indicates that flexibility in the initial fit between enzyme and substrate is sufficient to accommodate small changes in substrate size. The second step in the reaction, Schiff base interchange, may in fact show some change in rate with substrate structure, but this change must be considerably smaller than that shown by the decarboxylation step. Apparently the flexibility in the enzyme–substrate complex is to some extent maintained in the Schiff base interchange step, perhaps as a result of the fact that in this step some rotational freedom is maintained in the bonds between the 4 and 4′ carbons of the coenzyme and between the α carbon and the amino nitrogen of the substrate. This flexibility is lost in the decarboxylation step because the product is the highly conjugated quinoid intermediate. As first suggested by Dunathan,[14] the conformation required for decarboxylation places the carboxyl group substantially out of the plane of the conjugated system. The presence of substantial quinoid character in the transition state for decarboxylation places strong geometrical constraints on this step which were not present in earlier steps. Because of the extended conjugation, the system is no longer able to accommodate readily to changes in the distance between the distal binding group and the α carbon.

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Carbon Magnetic Resonance Spectroscopy on Carbon-13-Labeled Uracil in 5S Ribonucleic Acid[1]

Sir:

The carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra of the ^{13}C-enriched C-4 uridine carbons in 5S ribosomal ribonucleic acid (5S RNA) of *Salmonella typhimurium*, strain JL-1055,[2] has been obtained. The 5S RNA was isolated from a 5S RNA-tRNA mixture using gel permeation chromatography on Sephadex G-100 Superfine (Pharmacia Fine Chemicals). Gel permeation chromatography was also used to check the purity and the molecular size of the 5S RNA against suitable standards. The details of the separation along with the procedures for the incorporation of the ^{13}C label and the recovery of the low molecular weight RNA's are given elsewhere.[3,4] The ^{13}C-enrichment level was determined by mass spectroscopy to be 46%. The 5S RNA (34 mg) was dissolved in 370 μL of sterile 0.04 M $MgCl_2$, 5 mM dithiothreitol (DTT), 2mM EDTA solution. Proteinase K (0.2 mg) (EM Laboratories, Inc.) was added to destroy residual nuclease activity. The pH was adjusted to 7.4 with sterile 0.1 M NaOH. Dioxane (2%) was added for an internal reference and the sample placed in a 5-mm NMR sample tube. Deuterated

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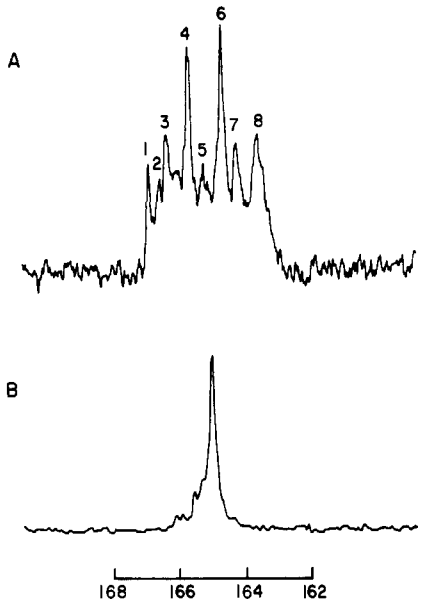

Figure 1. Carbon-13 NMR spectra of the C-4 uridine carbons in 5S RNA at (A) 37 °C with 18 000 transients, 0.25-Hz digital resolution, 4-s cycle time, 60° pulse width, and 2-s digital broadening, and at (B) 75 °C with 14 000 transients, 1.0-Hz digital resolution, 2-s cycle time, 90° pulse width, and 0.4-s digital broadening.

benzene in a 2-mm Wilmad WGS-5BL coxial insert was used for a field frequency lock.

The proton decoupled ^{13}C NMR spectra were taken on a variable-temperature Varian XL-100-15 NMR spectrometer equipped with a Fourier transform accessory, a Gryocode decoupler, and a Varian 620/f computer. Chemical shifts were measured relative to dioxane and referenced to Me_4Si by adding 66.3 ppm to the dioxane shifts. Two ^{13}C NMR spectra were obtained, the first at 37 °C with the molecule in its native state and the second after the sample was denatured with heat at 75 °C.

The spectra of the C-4 uridine carbons in 5S RNA at 37 and 75 °C are shown in Figure 1 and the chemical shifts of these carbons are listed in Table I. No lines belonging to the modified uridine bases found in tRNA were seen in the 5S RNA spectrum. This is expected since the sequence of the similar *Escherichia coli* 5S RNA does not contain any modified uridine bases.[5] The most striking feature of the 5S RNA spectrum (Figure 1A) is the large number of well-resolved lines in the uridine band covering a chemical shift range of ~3.6 ppm. A similar effect was observed in unfractionated tRNA, however; because of the heterogeneous nature of the sample, the individual lines were not so well resolved.[3] It should be noted that, while nucleic acids are known to form aggregates at the concentration used in this study, concentration studies have shown no indication that aggregation leads to additional resonances.[6] Upon heating to 75 °C the uridine band is collapsed to a single line at 165.1 ppm as a result of the random coil motion of the molecule making all of the uridines equivalent. The small downfield lines in Figure 1B are assumed to be due to incomplete unfolding. The chemical shift of the major line is very close to the 165.3 chemical shift reported for the tRNA uridine line at 82 °C.[3] Following heating to 75 °C, a third spectrum (not included) was obtained at 37 °C. This spectrum indicated that the 5S RNA had again assumed a secondary structure. However, the appearance of the spectrum indicated that the molecules did not go back to their original configuration.

The individual uridine lines observed in the 5S RNA spectrum, like those in the tRNA spectrum,[3] are a result of the differing chemical environments experienced by the individual uridines in the polynucleotide chain in its native state. The

Table I. Chemical Shifts, Integrals, and Uridine Counts of C-4 ^{13}C-Labeled Uracil in 5S RNA

Temp, °C	Line[a]	Chemical shift[b]	Integral[c]	Uridine count[d]
37	1	167.0	6	1
	2	166.8	6	1
	3	166.4	9	2
	4	165.8	19	4
	5	165.3	6	1
	6	164.8	23	5
	7	164.3	12	2
	8	163.7	19	4
75		165.1		

[a] Line numbers are those given in Figure 1A. [b] Chemical shifts are referenced to Me_4Si. [c] Integrals are percentage of each line to the total integral. [d] Uridine counts are estimates of the number of uridines in each line based on a total of 20 uridines for the molecule (see text).

nucleic acids in their native states fold over on themselves allowing complimentary regions to form base pairs and assume a double helical structure. The bases in these helical regions are held in comparatively fixed positions relative to their neighbors by base-pairing and base-stacking interactions with the result that the proton and carbon chemical shifts in these bases are strongly influenced by their neighbors. Once the correct sequence of *S. typhimurium* 5S RNA is known and the correct assignment of the lines in Figure 1A to their respective uridines is made, then the carbon-13 data may provide information on the secondary structure of 5S RNA. This is of interest because none of the several models proposed for the secondary structure of 5S has yet won general acceptance.[7-9]

With regard to evaluating the effects of the base-stacking interactions on chemical shifts, good agreement has been obtained between experiment and theory for proton lines considering only the effect of the circulating ring currents in the adjacent bases.[10] However, the application of this simple theory to carbon shifts has not yet succeeded,[11,12] suggesting that carbon lines are sensitive to more effects than proton lines. Charge-transfer effects have been shown to shift carbon lines by 1.4 ppm in simple trinitrobenzene–aromatic compound complexes[13] and may be making significant contributions to the ^{13}C shifts in these nitrogen bases.

The spectrum at 37 °C (Figure 1A) was integrated by means of spectrometer integration using standard Varian software and the integrals of each line were used to compute an estimated carbon count for each transition. The carbon count is based on the assumption that *S. typhimurium* 5S RNA, like *E. coli* 5S RNA, has 20 uridines in its sequence.[5] The values of these integrals and the carbon counts are listed in Table I. The data in Figure 1 and Table I are sufficient to allow an estimate of the amount of secondary structure in 5S RNA to be made. The uridine line at 75 °C lies between lines 5 and 6 in the 37 °C spectrum. Based on this similarity of shift positions, one of these two lines can be used to estimate the maximum number of uridines which could be assigned to the random coil portions of the molecule if the remaining shifts can be ascribed to secondary structural effects. The number of uridines involved in secondary interactions is estimated to be at least 75% and may be as high as 95% if line 5 represents a noninteracting uridine. This value corroborates the 65–75% value reported by Marshall and Smith using ^{19}F NMR on 5-fluorouridine in *E. coli* 5S RNA.[14]

Marshall and Smith[14] have outlined the desirable features of using ^{19}F-labeled compounds for studying macromolecules. However, the 1.2-ppm ^{19}F line widths which they observed are an order of magnitude larger than the 0.12-ppm ^{13}C line widths shown in Figure 1A. The narrower ^{13}C lines provide considerably more data from which information about the secondary

structure and other features in a biomolecule may be obtained. Because nonprotonated carbon lines tend to remain narrow even in molecules of considerable size, selective ^{13}C enrichment of these sites holds the promise of revealing many more subtle features of the secondary and tertiary interactions in biomolecules. In addition to the superior resolution, ^{13}C-labeling techniques also can claim the advantage of using atomic labels which are normally found in living systems.

References and Notes

(1) This work was supported in part by the National Institutes of Health of the U.S. Public Health Service Awards RR07092 and GM 08521. The synthesis of the carbon-13-labeled uracil was supported by ERDA Award E(11-1)-2451. One of the authors (W.D.H.) is indebted to the National Science Foundation for support in the form of a predoctoral fellowship. We wish to thank Dr. W. James Horton for synthesizing the carbon-13 uracil, Dr. James McClosky for the mass spectroscopic analysis, and Dr. John Ingraham for supplying the *Salmonella typhimurium* JL-1055 culture.
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Synthesis and Characterization of $[(\eta^5\text{-}C_5H_5)V(CO)_3H]^-$, a New Anionic Vanadium Carbonyl Hydride, and a Study of Its Reduction Reaction with Organic Halides. Observation of a Free-Radical Chain Process Having an Extremely Rapid Metal-to-Carbon Hydrogen Transfer Step

Sir:

We wish to report the preparation and certain reactions of a new anionic vanadium carbonyl hydride,[1,2] $[(\eta^5\text{-}C_5H_5)V(CO)_3H]^-$ (**1**), which may be prepared from commercially available[3] $(\eta^5\text{-}C_5H_5)V(CO)_4$. We have found that **1**, in THF or acetonitrile at room temperature, replaces halogen by hydrogen in a wide range of alkyl, vinyl, and acyl halides (Chart I). These reactions appear to proceed by a radical chain mechanism which involves an exceedingly high vanadium-to-carbon hydrogen transfer rate constant.

Treatment of a THF solution of $(\eta^5\text{-}C_5H_5)V(CO)_4$ with 0.7% Na/Hg yields the yellow salt $Na_2^+[(\eta^5\text{-}C_5H_5)V(CO)_3]^{2-}$, as has been reported,[4] but we have found it more convenient to titrate THF suspensions of sodium dispersion with $(\eta^5\text{-}C_5H_5)V(CO)_4$ to generate the dianion. Reaction with aqueous HCl gives the vanadium dimer[4] $(\eta^5\text{-}C_5H_5)_2V_2(CO)_5$, but addition of 1 equiv of water to a slurry of the dianion in THF yields a solution of $Na^+[(\eta^5\text{-}C_5H_5)V(CO)_3H]^-$ (Na-**1**). Addition of $(Ph_3P)_2N^+Cl^-[PPN^+Cl^-]$ precipitates NaCl, leaving a basic solution of PPN^+-**1**. Solid PPN^+-**1** is obtained by precipitation with petroleum ether (30–60 °C) and is purified by reprecipitation from THF upon very slow addition of petroleum ether. The overall yield based on $(\eta^5\text{-}C_5H_5)V(CO)_4$ is ~70%.

Chart I

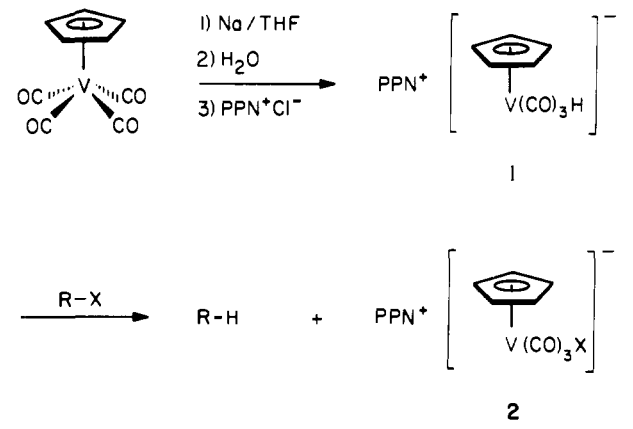
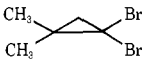
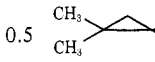
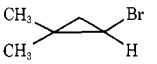
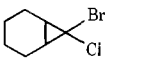
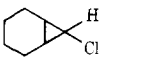


Table I. Interaction of Organic Substrates with $PPN^+[(\eta^5\text{-}C_5H_5)V(CO)_3H]^-$ at 25 °C in THF

Substrate	Reaction time, h	Product	Yield,[a] %
$n\text{-}C_8H_{17}Br$	4	$n\text{-}C_8H_{18}$	75
$n\text{-}C_6H_{13}Br$	7	$n\text{-}C_6H_{14}$	73
$n\text{-}C_{10}H_{21}Br$	24	$n\text{-}C_{10}H_{22}$	95
$c\text{-}C_6H_{11}Br$	10	$c\text{-}C_6H_{12}$	65
	0.5		95
	24	No reaction	
C_6H_5COCl	<0.1	C_6H_5CHO	40 (36[b])
$C_6H_5CH_2COCl$	<0.1	$C_6H_5CH_2CHO$	100[c]
$C_6H_{13}COCl$	<0.1	$C_6H_{13}CHO$	100[c]
$(CH_3)_3CBr$	12	$(CH_3)_3CH$	100
$C_6H_5CH_2Br$	<0.5	$C_6H_5CH_3$	90
$(-)\text{-}C_6H_5CH(Br)(CH_3)$	<0.5	$(\pm)\text{-}C_6H_5CHD(CH_3)$	64[d]
$C_4H_9CHBrCH_2Br$	2.5	$C_4H_9CH{=}CH_2$	100
C_6H_5Br	8.5	C_6H_6	43
$C_6H_5CH{=}CHBr$	5	$C_6H_5CH{=}CH_2$	46
$CH_3CH_2CH{=}(Br)CH_2CH_3$	>200	*cis*-3-Hexene	30[e]
		trans-3-Hexene	70[e]
$C_6H_5C(CH_3)_2CH_2Br$	48	$C_6H_5C(CH_3)_3$	83
	1		95
Cyclohexenone	1	No reaction	
$C_6H_{13}CO_2CH_3$	12	No reaction	

[a] Yields were determined by integration of NMR and gas chromatography peaks, except in cases indicated. [b] Isolated yield, *N,N'*-diphenylethylenediamine adduct. [c] Although spectroscopic monitoring indicates that yields of these aldehydes are essentially quantitative, we have so far experienced some difficulty isolating them. [d] Isolated (preparative VPC). [e] Corrected for unconverted starting material.

THF solutions of PPN^+-**1** exhibit two IR absorptions in the metal carbonyl region (1890 and 1780 cm^{-1}). The ^1H NMR spectrum in THF-d_8 shows a single resonance due to the cyclopentadienyl hydrogens at δ 4.57 ppm, and a very broad absorption centered[5] at δ −6.10 ppm due to the metal-bound hydrogen.

Table I gives the results of reduction experiments carried out on various organic halides, along with data on some unreactive substrates. Reactions were typically carried out at a 1:1 molar ratio of PPN^+-**1** to substrate by adding the organic halide to a THF solution of PPN^+-**1** at room temperature. The reactions may be easily monitored by observing the disap-

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