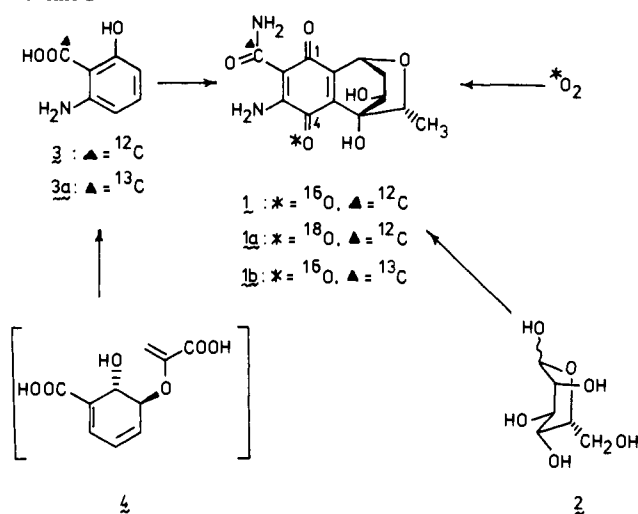**Scheme I**

0.04, 0.05, and 0.06 ppm), while the C-1 resonance showed one additional line shifted 0.03 ppm upfield. The multitude of lines for C-4 was due to the presence of NH_2, NHD, and ND_2 at C-3, as well as two definable orientations for the NHD due to hydrogen bonding. The unexpected shift for the C-1 resonance was due to a deuterium isotope effect from the amide hydrogen through hydrogen bonding to the carbonyl oxygen. Confirmation for an isotope shift through a hydrogen bond was obtained from an analogous experiment with partially exchanged methyl anthranilate, in which case the normal ester carbonyl was accompanied by two additional lines (upfield shifts of 0.01 and 0.02 ppm).[13] Therefore, we have concluded that the ^{18}O label from the biosynthetic experiment was located at C-4.

Although not previously a known natural product, we next tested 6-hydroxyanthranilic acid 3 as a logical precursor to the quinone ring. By use of an efficient four-step procedure,[14] [$^{13}COOH$]-6-hydroxyanthranilic acid (3a) was prepared, and 13-mg portions were added to each of three 200-mL production broths at 23, 35, and 47 h after they had been inoculated with a seed culture. Workup at 70 h afforded 33.8 mg of pure 1b. The 100-MHz ^{13}C NMR spectrum of 1b, obtained under identical conditions as for a natural abundance sample, showed a clean 7% enrichment in ^{13}C for the amide carbonyl (Figure 1C), demonstrating the intact incorporation of 3a.[15]

The results presented here reveal a new aromatic amino acid which would most reasonably be derived from the shikimate pathway via isochroismic acid (4),[16] as shown in Scheme I. This will be tested as part of our continuing studies of sarubicin biosynthesis.

(13) We are currently investigating how generally observable this phenomenon is and whether it can be used for structural assignments as well as measurements of hydrogen bond strengths. Transmission of a deuterium-induced isotope shift through a hydrogen bond in a disaccharide was recently reported: Christofides, J. C.; Davies, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099.

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(15) Based on the quantity (765 μmol) and enrichment (99%) of the 3a fed, and the quantity (124 μmol) and enrichment of 1b obtained pure, a minimum incorporation of 1.1% was obtained in the fermentation.

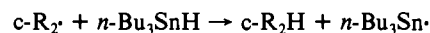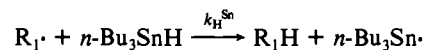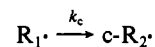(16) Haslam, E. "The Shikimate Pathway"; Halsted Press: New York, 1974; p 94, 96–97.

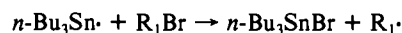# Absolute Rate Constants for Reaction of Phenyl, 2,2-Dimethylvinyl, Cyclopropyl, and Neopentyl Radicals with Tri-n-butylstannane. Comparison of the Radical Trapping Abilities of Tri-n-butylstannane and -germane[1]

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Intramolecular radical cyclizations have become important in organic synthesis because the generally high regio- and stereo-selectivities are complemented by the fact that there is no need to "protect" many types of functional groups.[4–7] For cyclizations involving carbon-centered radicals the usual procedure involves a free-radical chain reaction between a suitable precursor (e.g., a bromide) and tri-n-butylstannane:

$$n\text{-}Bu_3Sn\cdot + R_1Br \rightarrow n\text{-}Bu_3SnBr + R_1\cdot$$

$$R_1\cdot \xrightarrow{k_c} c\text{-}R_2\cdot$$

$$R_1\cdot + n\text{-}Bu_3SnH \xrightarrow{k_H^{Sn}} R_1H + n\text{-}Bu_3Sn\cdot$$

$$c\text{-}R_2\cdot + n\text{-}Bu_3SnH \rightarrow c\text{-}R_2H + n\text{-}Bu_3Sn\cdot$$

The yield of cyclized product, $c\text{-}R_2H$, is dependent on the concentration of tin hydride and on the ratio of the rate constants for hydrogen abstraction by $R_1\cdot$ and for its cyclization, i.e., k_H^{Sn}/k_c. Detailed synthetic strategies can be improved by kinetic information regarding the reactions involved and, in particular, by a knowledge of the magnitude of k_H^{Sn}. Absolute rate constants for this reaction have been measured for $R_1\cdot$ = methyl, n-alkyl, sec-alkyl, and tert-alkyl radicals by the rotating sector method at room temperature[8] and, more reliably and over a range of temperature, by the laser flash photolytic technique.[9] The growing importance of cyclizations involving carbon-centered radicals that are considerably more reactive than the alkyls, e.g., phenyl-,[10,11] vinyl-,[12–14] and cyclopropyl-centered[15] radicals, has generated a need for absolute values of k_H for these classes of radicals.

Results and Discussion. Kinetic data for phenyl, 2,2-dimethylvinyl, and cyclopropyl radicals were obtained by monitoring the growth of the $n\text{-}Bu_3Sn\cdot$ radical at 400 nm[9,16] following the

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Communications to the Editor

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Table I. Kinetic Parameters for Reactions of Some Carbon-Centered Radicals with Tri-*n*-butylstannane[a]

radical	temp range, K	log $(A/M^{-1} s^{-1})$	E, kcal/mol	$10^{-7}k_H^{Sn}$, $M^{-1} s^{-1}$ (at 30 °C)
C_6H_5·[b]	280–332	10.0 ± 0.2	1.7 ± 0.3	59
$(CH_3)_2C=CH$·[c]	249–298	9.7 ± 0.3	1.6 ± 0.4	35
c-C_3H_5·[b]	256–318	9.3 ± 0.5	1.9 ± 0.6	8.5
CH_3·[d]	231–317	9.4 ± 0.3	3.2 ± 0.3	1.2
RCH_2CH_2·[d,e]	245–355	9.1 ± 0.3	3.7 ± 0.3	0.27
$(CH_3)_3CCH_2$·[f]	244–300	8.5 ± 0.2	2.7 ± 0.2	0.36
$(CH_3)_2CH$·[d]	251–358	8.7 ± 0.4	3.5 ± 0.5	0.15
$(CH_3)_3C$·[d]	263–351	8.4 ± 0.1	3.0 ± 0.2	0.17

[a] Errors in this table and in the text represent 95% confidence limits but include only random errors. [b] This work; benzene solvent. [c] This work; *n*-pentane solvent. [d] Reference 9. [e] Combined data for R = H (ethyl) and R = CH_3CH_2 (*n*-butyl). [f] This work; isooctane solvent.

Table II. Absolute Rate Constants for Reactions of Some Carbon-Centered Radicals with Tri-*n*-butylgermane at Ambient Temperatures

radical	T, °C	$10^{-6}k^{Ge}$, $M^{-1} s^{-1}$	k_H^{Sn}/k_H^{Ge}
C_6H_5·[a]	29	260 ± 28	2
$(CH_3)_2C=CH$·[b]	27	35 ± 5	10
c-C_3H_5·[b]	30	13 ± 2	7
CH_3·[c]	27	0.5	20
RCH_2CH_2·[d,e]	27	0.1	24

[a] This work; benzene solvent. [b] This work; *n*-pentane solvent. [c] Reference 30. [d] Reference 31. [e] R = $CH_2=CHCH_2H_2$ (5-hexenyl); log $(A/M^{-1} s^{-1})$ = 8.4 ± 0.5, E = 4.7 ± 0.6 kcal/mol.

308-nm laser flash photolysis of the appropriate acyl or aroyl peroxide,[17] $R_1CO_2CO_2R_1$. The results are summarized in Table I.

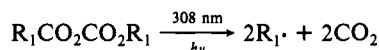$$R_1CO_2CO_2R_1 \xrightarrow[h\nu]{308\ nm} 2R_1· + 2CO_2$$

Table I also includes new kinetic data for reaction of neopentyl with *n*-Bu₃SnH, since radicals of the general class $RCMe_2CH_2$· are finding increasing use as mechanistic probes.[23–29] It is therefore important to know whether or not such radicals have the same reactivity toward the stannane as do primary alkyl radicals. Our earlier laser flash data on alkyl radicals[9] has been included in Table I for ready reference.

Tri-*n*-butylgermane is a considerably poorer hydrogen donor to alkyl radicals than tri-*n*-butylstannane.[30,31] This does not necessarily detract from its synthetic utility as an alternative to the tin hydride. In the first place, the *n*-Bu₃Ge· radical is at least as good a halogen abstracting agent as *n*-Bu₃Sn·,[32] and in the

second place, a less reactive hydrogen donor could be an advantage in cases where the cyclization is rather slow. Kinetic data for hydrogen abstraction from *n*-Bu₃GeH by reactive carbon-centered radicals was also obtained by laser flash photolysis of $R_1CO_2CO_2R_1$.

$$R_1· + n\text{-}Bu_3GeH \xrightarrow{k_H^{Ge}} R_1H + n\text{-}Bu_3Ge·$$
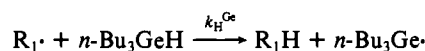
Growth of the *n*-Bu₃Ge· radical[32,33] (at 300 nm) was used to measure k_H^{Ge} for the 2,2-dimethylvinyl radical. Since this growth is considerably more difficult to monitor than that of *n*-Bu₃Sn·, we employed the probe technique[32,34] (using benzene as our probe) for measurement of k_H^{Ge} for phenyl (at 340 nm) and cyclopropyl (at 322 nm) radicals. Our measurements of k_H^{Ge} have been confined to room temperature. These data are summarized in Table II together with data from the literature.

Our kinetic data yield no surprises. That is, k_H^{Sn} and k_H^{Ge} values decrease with a decrease in the R_1–H bond strength[35,36] and, for any specific radical, $k_H^{Sn} > k_H^{Ge}$. From the data for the 1,5-cyclization of the 2,2-dimethylhex-5-en-1-yl radical to form the (2,2-dimethylcyclopentyl)carbinyl radical[24] we calculate the following Arrhenius parameters for this cyclization, log (A_c/s^{-1}) = 9.9, and E_c = 4.4 kcal/mol, which yield k_c = 5.3 × 10⁶ s⁻¹ at 30 °C. Comparison with our earlier data on the ring closure of hex-5-en-1-yl, viz.,[9] log (A_c/s^{-1}) = 10.37 ± 0.32, E_c = 6.85 ± 0.42 kcal/mol, and k_c = 2.7 × 10⁵ s⁻¹ at 30 °C, confirms earlier reports[23,24] that 2,2-dimethyl substitution accelerates cyclization, presumably because of the Thorpe–Ingold (*gem*-dimethyl) effect.

Of greater interest would be the calculation of k_c values for the highly reactive radicals but unfortunately kinetically useful data are extremely limited. Beckwith and Gara[11] have reported on some aryl radical cyclizations at 130 °C, and by using essentially the same method we have now obtained more extensive data over the temperature range 0–150 °C for the reactions

X · CH_2, O
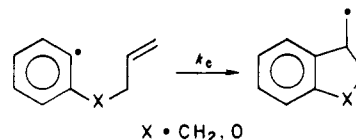
When X = CH_2, log $[(k_c/k_H^{Sn})/M]$ = (1.2 ± 0.1) − (1.9 ± 0.2)/θ where θ = 2.3RT kcal/mol; for this cyclization therefore, log (A_c/s^{-1}) = 11.2, E_c = 3.6 kcal/mol, and k_c = 4.0 × 10⁸ s⁻¹ at 30 °C. When X = O, log $[(k_c/k_H^{Sn})/M]$ = (2.1 ± 0.1) − (1.5 ± 0.1)/θ and hence, log (A_c/s^{-1}) = 12.1, E_c = 3.2 kcal/mol, and k_c = 6.3 × 10⁹ s⁻¹ at 30 °C, so that this particular ring closure is the fastest yet observed. The enhanced rates of cyclization of alkenylaryl radicals compared with the corresponding alkenyl radicals is in agreement with the relative energies of appropriate model transition structures calculated by force field methods.[37]

Although no kinetically useful data are available regarding the intramolecular reactions of vinyl-centered radicals, we note two reports on cyclopropyl-centered radicals.[15,38] For the reaction[15]
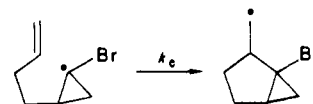
we can estimate that k_c/k_H^{Sn} is probably ca. 1.1 M at 80 °C,

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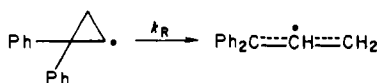
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hence[39] $k_c \approx 1.5 \times 10^8$ s^{-1} at this temperature. For the reaction[38]

we estimate that $k_R/k_H{}^{Sn} \approx 0.03$ M at 78 °C, hence[39] $k_R \approx 4 \times 10^6$ s^{-1} at this temperature.

We are confident that the kinetic data summarized in Tables I and II will find wide and general use in mechanistic and synthetic studies.

Experimental Section. Tri-*n*-butylstannane was purchased from Aldrich and used as received. Tri-*n*-butylgermane was prepared by reduction of tri-*n*-butylgermanium chloride by lithium aluminum hydride.[40,41] Benzoyl peroxide was obtained from Aldrich and purified by standard methods. Bis(cyclopropylformyl) peroxide[42] and 3,3-dimethylacryloyl peroxide[43] were prepared by literature methods.

Laser Flash Photolysis Experiments. Samples (2 mL, 0.2 M peroxide) were contained in 7 × 7 mm^2 cells made of Suprasil and were deoxygenated by purging with oxygen-free nitrogen. A Lumonics TE-860-2 excimer laser (Xe–HCl–He mixture) with pulses at 308 nm (~4 ns, up to 80 mJ/pulse) was used for sample excitation. Further details on the laser flash apparatus have been given elsewhere.[44]

Registry No. *n*-Bu$_3$Sn·, 688-73-3; *n*-Bu$_3$Ge·, 998-39-0; C$_6$H$_5$·, 2396-01-2; (CH$_3$)$_2$C=CH·, 33825-33-1; *c*-C$_3$H$_5$·, 2417-82-5; (CH$_3$)$_3$CCH$_2$·, 3744-21-6.

Supplementary Material Available: Tables III–IX giving detailed kinetic data (11 pages). Ordering information is given on any current masthead page.

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Nature of the Cadmium Sites in Rat Liver Metallothionein 1 from Cd K-Edge EXAFS

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The Metallothioneins are a unique and widely distributed group of metal binding proteins. They are characterized by their low molecular weight (ca. 6000), high cysteinyl content, and ability to bind substantial numbers of a wide variety of metal ions. The majority of mammalian metallothioneins may be separated by ion-exchange chromatography into two main isoforms, designated as metallothioneins 1 and 2, which differ in their overall negative charge and details of their amino acid composition. However, the number and position of the cysteinyl residues in the polypeptide chain is highly conserved with the sequence -Cys-A-Cys- being predominant.[1] Although crystals of metallothionein have been

isolated, an X-ray crystal structure of the protein has not yet been reported and the information concerning the nature of the metal sites has been obtained from various spectroscopic studies.[2] The general consensus is that the metal atoms are bound by the cysteinyl sulfurs and this has been confirmed for Zn(II)[3] and Cu(I)[4] from K-edge EXAFS data. [113]Cd NMR spectroscopy has been very informative concerning the nature of Cd-metallothioneins.[5-9] The occurrence of the resonances at 600–700 ppm downfield of Cd(ClO$_4$)$_2$ has generally been taken as indicative of Cd(Scys)$_4$ coordination. Data for [113]Cd-enriched samples not only clearly indicate different Cd sites but also demonstrate the existence of close Cd···Cd approaches. Furthermore, the information obtained from [113]Cd-decoupling experiments led Otvos and Armitage to propose[8] that Cd$_7$-metallothionein contains two distinct metal aggregates, involving four and three metal atoms in which each metal is tetrahedrally coordinated by four cysteinyl sulfurs; all 20 cysteinyl residues are involved as terminal or μ_2 ligands. These [113]Cd NMR studies have been very influential in respect of the considerations of the binding of other metals by metallothioneins.

Therefore, we considered that it was important to provide alternative structural information concerning the environment of Cd in metallothionein. The availability of a significant X-ray flux at the wavelength of the cadmium K-edge ($\mu = 0.46$ Å) from the Wiggler magnet of the synchrotron radiation source (SRS) at the Daresbury Laboratory has enabled the X-ray absorption spectrum of cadmium in rat liver metallothionein 1 to be recorded. This is the first time the nature of a cadmium site in a biological system has been probed by X-ray absorption spectroscopy and the results obtained are complementary to the [113]Cd NMR data obtained for this protein.[5-9]

Rat liver Cd/Zn-metallothionein 1 was isolated from rats injected intraperitoneally when an aqueous solution of CdSO$_4$ (0.5 mg of Cd per kg of body weight on day one, followed by five injections of 2.0 mg of Cd per kg of body weight on alternate days). Metallothionein 1 was purified,[10] by a combination of gel filtration and ion exchange chromatography, and freeze-dried. The product was electrophoretically homogeneous and contained 7 mol of metal/mole of protein with a Cd:Zn ratio of 1.3:1. Fully substituted Cd$_7$-metallothionein was prepared by treating an aqueous solution of the Cd/Zn-metallothionein-1 with a 10-fold molar excess of CdSO$_4$. After incubation for 12 h at 1 °C, the protein was separated from Cd^{2+} and Zn^{2+} by gel filtration on Sephadex G.25, using distilled water as eluant, and then freeze-dried. The product contained no Zn.

Samples of freeze-dried rat liver Cd/Zn-metallothionein 1, Cd-metallothionein 1, and finely ground CdS diluted with boron nitride were mounted in sample cells of an aluminum sheet 1-mm thick and 1.5 × 2 cm in cross-section, in which a window 3 × 15 mm had been cut; each sample was contained between Sellotape windows. The cell was mounted in an evacuated cryostat and the sample cooled to ca. 77 K. X-ray absorption spectra in the transmission mode were obtained on the EXAFS station of the beam-line of the 4.5 T Wiggler magnet at the SRS. During the experiments, the SRS was operating at an energy of 1.8 GeV with an average current of 180 mA. A channel-cut Si 220 crystal

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