utilized in stabilizing unusual structures or interactions in certain polynucleotides. Quite independent of whether certain biological systems naturally make use of these interesting binding properties, the results presented here provide data which will be needed in ESR studies of the interaction of copper with polynucleotides. They also suggest ways in which pH may be used as an additional parameter to control the nature of the complexes formed.

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Communications to the Editor

Rate Constants for Spin Trapping. Primary Alkyl Radicals[1]

Sir:

The EPR spectroscopic technique of "spin trapping" has been used qualitatively to detect and identify transient free radicals for several years.[2] Its quantitative use in mechanistic studies has been hampered by the paucity of data on the rate constant, k_1, for the reactions of the radicals with the commonly employed spin traps.

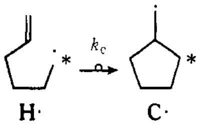
$$\mathrm{R}\cdot + \mathrm{T} \xrightarrow{k_1} \mathrm{RT}\cdot \qquad (1)$$

What little rate data is available[3-9] rests on competition experiments with reactions having "known" rate constants. However, these "known" rate constants are themselves uncertain to varying degrees.[10] In view of the great potential of spin trapping, we have begun a program to determine accurate rate constants for the trapping of some commonly encountered radicals.

The 5-hexenyl radical, **H·**, isomerizes to cyclopentylmethyl, **C·**, at a rate which is reliably known.[11,12] Since both radicals are primary alkyls, the spin adducts that they form with a spin trap, **T**, will have very similar properties, i.e., similar EPR spectra and similar kinetic and thermodynamic stabilities. A nice distinction between the spectra of the two spin adducts, **HT·** and **CT·**, can be obtained by labeling the 5-hexenyl radical with C-13 in the 1 position, since hyperfine splitting (hfs) by this carbon will only be detectable in **HT·**. Any further reactions of **HT·** and **CT·** should have virtually equal rate constants and so the ratio of [**HT·**]/[**CT·**] should be independent of reaction time *unless* spin trapping is reversible, in which case this ratio will decrease as the reaction proceeds. The rate constant for trapping, k^{T}, can be calculated from the relation

$$k^{\mathrm{T}} = k_{\mathrm{c}}[\mathbf{HT\cdot}]/[\mathrm{T}][\mathbf{CT\cdot}]$$

provided the spin–adduct ratio is extrapolated to zero time if it shows any variation.



H· C·

$$\mathbf{H}\cdot + \mathrm{T} \xrightarrow{k^{\mathrm{T}}} \mathbf{H}\mathrm{T}\cdot$$
$$\mathrm{C}\cdot + \mathrm{T} \longrightarrow \mathrm{CT}\cdot$$

We have applied this procedure to phenyl-*N*-*tert*-butyl nitrone (PBN) and to 2-methyl-2-nitrosopropane (NtB)[13] using di([2-^{13}C]-6-heptenoyl) peroxide (90 at. % ^{13}C) as a *thermal* source of [1-^{13}C]-5-hexenyl in benzene at 40 °C. The ^{13}C hfs constants of the labeled **HT·** are 3.1 G, for T = PBN[14] and 5.0 G, for NtB;[15] so the lines due to **HT·** are resolved from those of **CT·** in both cases. For one trap, NtB, the [**HT·**]/[**CT·**] ratio decreased slightly with time (~20% between $t = 5$ and $t = 30$ min) suggesting that the addition is marginally reversible at 40 °C.[16] Using ~0.06 M peroxide and [PBN] = 0.44 and 0.30 M[18] and extrapolating to $t = 0$ gave $k^{\mathrm{T}}_{\mathrm{PBN}}/k_{\mathrm{c}} = 0.75$ and 0.75, respectively, and with [NtB] = 0.032 and 0.026 M,[17] $k^{\mathrm{T}}_{\mathrm{NtB}}/k_{\mathrm{c}} = 50.9$ and 50.4, respectively. At 40 °C, $k_{\mathrm{c}} = 1.7_8 \times 10^5\ \mathrm{s}^{-1}$;[12] therefore $k^{\mathrm{T}}_{\mathrm{PBN}} = 1.3_4 \times 10^5\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ and $k^{\mathrm{T}}_{\mathrm{NtB}} = 90.2 \times 10^5\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. That the rate constant ratio, $k^{\mathrm{T}}_{\mathrm{NtB}}/k^{\mathrm{T}}_{\mathrm{PBN}} = 90.2/1.3_4 = 67.4$ is correct was confirmed by competitive experiments using the *n*-hexyl radical (from *n*-heptanoyl peroxide) in the presence of both spin traps. At

Table I. Steady-State Concentration of *n*-Hexyl-*tert*-butyl Nitroxide Generated from 6.6 × 10^{-2} M *n*-Heptanoyl Peroxide and NtB in Benzene at 40 °C

[NtB] × 10^3, M	$[RT\cdot]_{ss}$ × 10^6, M	$(k_1[T]/[RT\cdot]_{ss})$ × $10^{-9\,a}$	$2k_3^{a,b}$
28.4	11.6	21.8	140
14.2	13.0	9.72	103
5.67	10.8	4.67	128
2.84	10.8	2.34	94
0.57	5.0	1.01	158
0.28	3.2	0.78	132

[a] In M^{-1} sec^{-1} units. [b] Calculated from eq 4 using $v_i = 2 \times 10^{-8}$ M s^{-1} and $k_2 = 6.8 \times 10^8$ M^{-1} s^{-1} which gives the minimum variation in $2k_3$.

[PBN]/[NtB] ratios of 24 and 32 the $k^T{}_{NtB}/k^T{}_{PBN}$ ratios extrapolated to $t = 0$ were 64.8 and 60.8, respectively.

Additional rate constants can be readily obtained from experiments of this type. For example, it has previously been shown[3] that the rate of decomposition of a peroxide, v_i, can be obtained by monitoring the initial rate of formation of the spin adduct. However, it has not previously been pointed out that with most traps it should be possible to use the steady-state concentration of the adduct to determine the rate constants for the two processes that normally will lead to its consumption, viz.,

$$R\cdot + RT\cdot \xrightarrow{k_2} \text{products} \qquad (2)$$

$$RT\cdot + RT\cdot \xrightarrow{2k_3} \text{products} \qquad (3)$$

For initiation and reactions 1-3, the appropriate equation is

$$\left(\frac{k_1[T]}{[RT\cdot]_{ss}} - k_2\right) \Big/ \left(\frac{k_1[T]}{[RT\cdot]_{ss}} + k_2\right) = \frac{2k_3}{v_i}[RT\cdot]_{ss}{}^2 \qquad (4)$$

The data in Table I illustrate this application of spin trapping for R = *n*-hexyl and T = NtB in benzene at 40 °C. Measurement of the rate of RT· formation gave $v_i = 2 \times 10^{-8}$ M s^{-1}.[19] With this value the tabulated data could best be fitted to equation 4 by taking $k_2 = 6.8 \times 10^8$ M^{-1} s^{-1} which gave $2k_3$ 125 ± 20 M^{-1} s^{-1}. The value found for k_2 is in the range that has been found for addition of other alkyls to sterically hindered dialkyl nitroxides such as 2,2,6,6-tetramethyl-4-oxopiperidine-*N*-oxyl,[20-23] e.g., 4 × 10^8 M^{-1} s^{-1} for the cyclopentyl addition.[23,24] The value found for $2k_3$ was checked by direct measurement of the rate of decay of *n*-hexyl-*tert*-butyl nitroxide. This radical was generated from its hydroxylamine by photolysis in benzene in the presence of di-*tert*-butyl peroxide.[25] The exact value of $2k_3$ could not be determined because we were unable to prepare the *N*-*n*-hexyl-*N*-*tert*-butylhydroxylamine completely free from *N*,*N*-di-*tert*-butylhydroxylamine and di-*tert*-butyl nitroxide.[26] The effect of these impurities is to accelerate the decay of *n*-hexyl-*tert*-butyl nitroxide. However, under conditions of minimum impurity we were able to establish that *n*-hexyl-*tert*-butyl nitroxide decayed with second-order kinetics and that $2k_3 \leq 246 \pm 47$ M^{-1} s^{-1} at 40 °C.

This work is currently being extended to other spin traps and other temperatures.

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(1) Issued as N.R.C.C. No. 16177.

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(9) In brief: $C_6H_5C(O)O\cdot$ + PBN, $k_1 = (1–10) \times 10^5$ M^{-1} s^{-1}, at 40 °C in benzene;[3,5] $(CH_3)_3CO\cdot$ + PBN, $k_1 = 55 \times 10^5$, $(CH_3)_3CO\cdot$ + NtB, $k_1 = 15 \times 10^5$, both at 25 °C in benzene;[4] $C_6H_5\cdot$ + PBN, $k_1 = 120 \times 10^5$, at 25 °C in methanol;[5] $(CH_3)_3CO\dot{C}O$ + NtB, $k_1 = (11–55) \times 10^5$, $[(CH_3)_3CO]_2\dot{C}H$, $k_1 = (9–45) \times 10^5$, both at 40 °C in di-*tert*-butyl peroxide;[6,7] $CH_3O\cdot$ + NtB, $k_1 = 1.3 \times 10^6$, at −45 °C in methanol.[8]

(10) Some "known" rate constants have been estimated from data obtained under very different conditions, sometimes on rather flimsy grounds. Even the most reliable of these "known" rate constants (i.e., that for $2(CH_3)_3C\cdot \rightarrow$ products which was used in ref 6 and 7) may be uncertain under the trapping conditions by a factor of 5 (see footnote, p 81, ref 7).

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(13) The acronym most often used. It stands for nitroso-*tert*-butane.

(14) Cf. 3.15 G for the $^{13}CH_3\cdot$ adduct: K. Sommermeyer, private communication.

(15) Cf. 4.38 G for $(Me_3{}^{13}C)_2NO\cdot$: R. Briere, H. Lemaire, and A. Rassat, *J. Chem. Phys.*, **48**, 1429 (1968).

(16) Unimolecular decompositions that may be more facile[17] such as,

$$RN(\dot{O})CMe_3 \rightarrow RN{=}O + Me_3C\cdot$$

and

$$C_6H_5CHRN(\dot{O})CMe_3 \rightarrow C_6H_5\dot{C}HR + Me_3CN{=}O$$

are not expected to affect the [**HT**·]/[**CT**·] ratio.

(17) See, e.g., O. W. Maender and E. G. Janzen, *J. Org. Chem.*, **34**, 4072 (1969).

(18) The range of concentrations available is limited by the need to obtain approximately equal concentration of the two radicals.

(19) This yields a rate constant for the decomposition of *n*-heptanoyl peroxide to free radicals of 3 × 10^{-7} s^{-1} in benzene at 40 °C, which is in good agreement with other data. See, e.g., J. E. Guillet, T. R. Walker, M. F. Meyer, J. P. Hawk, and E. B. Towne, *Ind. Eng. Chem., Prod. Res. Devel.*, **3**, 257 (1964).

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(26) Analogous problems have been reported by others; cf. G. Chapelet-Letourneux, H. Lemaire, and A. Rassat, *Bull. Soc. Chim. Fr.*, 3283 (1965).

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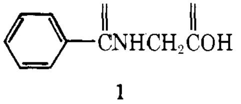
Aminomethaneboronic Acids. Synthesis and Inhibition of a Boron Analogue of Esterase Substrates

Sir:

We have synthesized a boronic acid analogue of hippuric acid (*n*-benzoylglycine) (**1**), an N-blocked amino acid, the esters and amides of which are used as substrates by proteolytic enzymes. The analogue synthesized (**2**) represents an example



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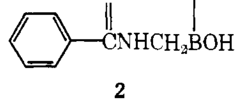

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of an amino acid substrate where the carboxyl carbon has been replaced by boron. Kinetic inhibition studies with α-chymotrypsin show that **2** is a potent, competitive inhibitor, and that it binds three orders of magnitude more tightly than does **1** to this enzyme. Our results suggest that this inhibitor acts as a possible analogue (**3**) of the metastable tetrahedral reaction