Round-Trip Radical Probes: Ring Cleavage of the Bicyclo[1.1.1]pentylcarbinyl Radical

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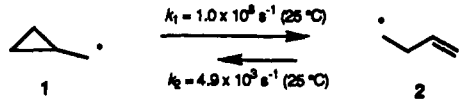
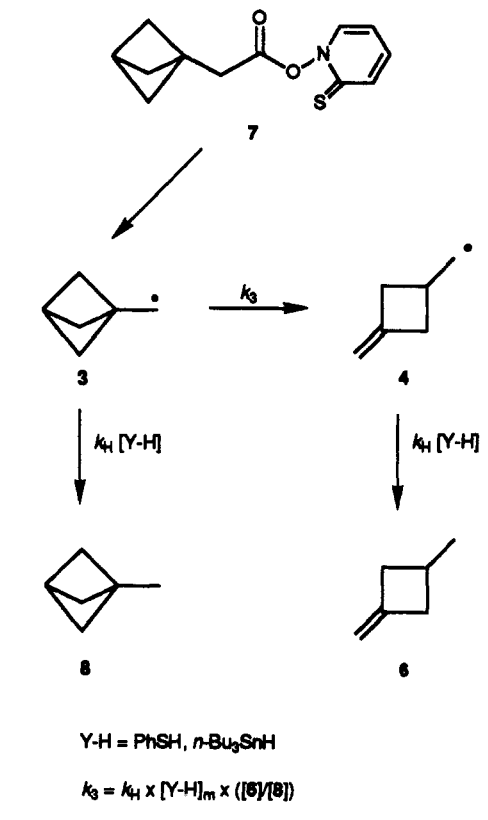
The novel concept of a round-trip probe for radical intermediates in reaction mechanisms is proposed and defined in this paper. A round-trip radical probe undergoes skeletal rearrangements such that the radical is returned to its site of origin. These probes should be especially useful for the study of enzyme mechanisms, since the special requirements of the active site may lead to ambiguous results using standard nonround-trip radical probes. The ring cleavages and rearrangements of the bicyclo[1.1.1]pentylcarbinyl radical are described as the prototypical round-trip radical probe. We have measured the rate constant for the ring opening of the bicyclo[1.1.1]pentylcarbinyl radical over the temperature range −42 to 60 °C and have determined a temperature-dependent function for the ring opening of log (k_3/s^{-1}) = 12.78 (±0.26) − 7.79 (±0.35)/Θ and a rate constant for ring opening of 1.15 × 10^7 s^{-1} at 25 °C.

Recently, there has been much interest in the detection of radical intermediates using "radical clocks",[2] such as the prototypic rearrangement of **1** to **2**.[3] We became interested in the general problem of detecting radical intermediates in enzyme-catalyzed reactions. Radical probes such as the rearrangement of **1** to **2** have been very useful for the detection of free radicals in solution. Probing for transient radical intermediates sequestered in enzyme active sites presents a more challenging problem, requiring new concepts and criteria in probe design. As an essential feature of catalysis, most enzyme active sites hold intermediates in specific, fixed, spatial orientations relative to the active site and relative to other substrates, intermediates, and products. An intermediate at an enzyme active site is tightly tied down and cannot freely rotate or tumble about in the active site, in contrast to the properties of reactive intermediates formed in solution which can exit and enter solvent cages and can freely tumble within solvent cages. To address the problem of nontumbling of radical intermediates in enzyme active sites we would like to propose the use of a new class of radical rearrangements, round-trip radical rearrangements. *We define a round-trip radical rearrangement as a radical-mediated skeletal isomerization which returns the radical to its original site, leaving a rearranged skeleton as diagnostic evidence that a radical intermediate had been formed.*



As a prototype round-trip radical rearrangement consider the isomerization of the bicyclo[1.1.1]pentylcarbinyl radical (**3**) to the cyclobuten-3-carbinyl radical (**4**) then on to the 1,4-pentadienyl-2-carbinyl radical (**5**). The ring opening of the bicyclo[1.1.1]pentylcarbinyl radical (**3**) is a two step process; homolysis of a β−γ bond to give the (3-methylenecyclobutyl)carbinyl radical (**4**), followed by the opening of the second ring to give the 1,4-pentadienyl-2-carbinyl radical (**5**). The second step in this process has recently been studied by Walton[5] employing kinetic ESR and the tin hydride method over the temperature range −62 to +127 °C and was found to have an Arrhenius function for ring opening of log (k_4/s^{-1}) = 12.9−11.5/Θ where k_4 = 2.80 × 10^4 s^{-1} at 25 °C. In this paper, we report the rate constant for the first step in this round-trip radical rearrangement, k_3.

Scheme I



Results

The ring opening of **3** to **4** was expected to be quite fast (>10^6 s^{-1}) due to the ring strain in **3**. In addition, the 3-fold symmetry of radical **3** should maximize the overlap of the SOMO with any of the three β−γ bonds that could be broken. The "PTOC-thiol" method described by Newcomb[3] for the measurement of fast radical rearrangements was well suited to this application. The *N*-hydroxypyridine-2-thione (PTOC) ester of bicyclo[1.1.1]pentylacetic acid[6] was prepared and used as the radical precursor.

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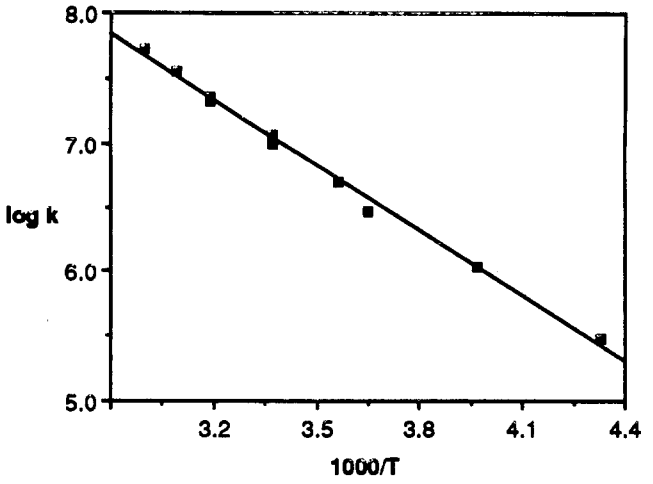

Figure 1. Arrhenius plot for the ring opening of **3**.

Thiophenol (k_H = 1.36 × 10^8 M^{-1} s^{-1} at 25 °C)[7] and *n*-Bu_3SnH (k_H = 3.29 × 10^6 M^{-1} s^{-1} at 25 °C)[8] were employed as radical traps with toluene as the solvent (Scheme I). Thiophenol was used as the H atom donor at temperatures ranging from 60 to 8 °C, but was too efficient a trap to be used at lower temperatures, as too little rearranged product would be trapped to give accurately measurable product ratios. Therefore *n*-Bu_3SnH, a less efficient H atom donor, was used for runs at 1, −21, and −42 °C. Thiophenol is known to react with primary, secondary, and tertiary radicals at approximately the same rate constant. Therefore, the rate constant for the reaction of thiophenol with a primary radical should be a good value for the reaction of thiophenol with **3**. However, *n*-Bu_3SnH reacts with neopentyl radical at a slightly faster rate than a primary radical, and the Arrhenius function for the reaction of *n*-Bu_3SnH with neopentyl radical was chosen as the standard in this case. The Arrhenius function (Figure 1) for the ring opening of **3** over the temperature range −42 to 60 °C was found to be log (k_3/s^{-1}) = 12.78 (±0.26) − 7.79 (±0.35)/Θ (r^2 = 0.993) where k_3 = 1.15 × 10^7 s^{-1} at 25 °C and the errors are 2σ.

Discussion

There are five important points that must be considered when using any radical rearrangement as a mechanistic probe. These points will be discussed in detail in the following paragraphs to illustrate the main advantages of a round-trip radical probe compared to a conventional non-round-trip radical probe for use in studies of enzyme reaction mechanisms.

(*1*) *What is the rate of the radical-probing reaction compared to that of normal catalysis?* When radical intermediates are properly oriented close together at the active site the rates of electron or proton transfer may approach the upper limit for chemical reactions, ~10^{13} s^{-1}. Even very fast radical probes will be much slower, but, if it is certain that all radical rearrangements result in diagnostic products (i.e., no leakage into other pathways; vide infra), then this problem simply becomes an analytical chemistry problem in detecting small yields of products using GC, GC–MS, and/or radioactive tracers.

(*2*) *Can radical intermediates tumble freely in enzyme active sites?* With a conventional non-round-trip radical probe the rearranged radical must turn around in the active site to complete a radical diagnostic reaction. A round-trip radical probe delivers the radical back to its site of origin and no active site tumbling of radical intermediates is necessary. Here, the round trip probe deals with a crucial issue that will always plague conventional non-round-trip probes.

(*3*) *What products should be taken as diagnostic evidence for radical intermediates?* With a conventional non-round-trip radical probe, the rearranged radical will often be a different type of radical than the original radical. Assumptions must be made about what chemistry the rearranged radical will undergo to form a product diagnostic of the radical intermediate, and there is always the chance that a mechanistically significant product will not be searched for because the investigator did not consider it. With a round-trip radical probe the rearranged radical is the same type of radical as the original radical. Returned to its site of origin, the rearranged radical can complete a normal reaction; the products diagnostic of radical intermediates are obvious and unambiguous.

(*4*) *Will the enzyme perturb the thermodynamics of radical rearrangement?* With a conventional non-round-trip radical probe the radical rearrangement may be thermodynamically favorable in a model reaction in solution, but it may not be favorable at the active site since radical intermediates should be stabilized by enzyme functional groups and by each other. For example, a radical cation and radical anion in the close proximity of the active site could conceivably stablize each other via coulombic interactions. With a round-trip radical probe the rearrangement returns the radical to its site of origin where it can experience the same enzymic radical-stabilizing effects that the enzyme focused on the original radical at the same site.

(*5*) *Will the enzyme perturb (slow down) the kinetics of radical rearrangement?* This problem remains for both conventional non-round-trip and round-trip radical probes. In principle, it can be resolved by using more sensitive methods of product detection because a slower rate of radical rearrangement simply means that less diagnostic

Table I. Kinetic Data for the Reaction of 7 with *n*-Bu_3SnH and PhSH in Toluene

T^a (°C)	$[Y-H]_m{}^b$ (M)	8/6	$k_3 \times 10^{-7}{}^c$ (s^{-1})
	(PhSH Trap)		
8	0.177	4.11	0.488
24	0.232	2.73	1.14
24	0.448	5.32	1.13
40	0.279	2.08	2.09
40	0.468	3.23	2.26
40	0.773	5.25	2.29
51	0.636	3.02	3.62
60	0.898	3.10	5.35
24[d]	1.32	18.2	0.97
24[d]	0.223	3.08	0.97
	(*n*-Bu_3SnH Trap)		
−42	0.554	1.67	0.0295
−21	0.563	0.739	0.107
1	0.551	0.414	0.293

[a] ±1 °C. [b] Mean concentration. [c] Evaluated using $k_3 = k_H \times [Y-H]_m \times ([6]/[8])$ where log (k_H, PhSH) = 9.41 (±0.13) − 1.74 (±0.21)/Θ[7] and log (k_H, *n*-Bu_3SnH) = 8.5 (±0.2) − 2.7 (±0.2)/Θ.[8] [d] Benzene-d_6 solvent.

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radical product is formed. Because of the superior features of round-trip radical probes, a search for very small quantities of diagnostic products can be undertaken with more confidence in obtaining unambiguous, interpretable results.

In addition to all of the intrinsic advantages of round-trip radical probes, there is the added advantage in the case of the bicyclo[1.1.1]pentylcarbinyl radical of the 3-fold symmetry axis, which should ensure that an enzyme cannot hold the transient radical in a conformation that stereoelectronically disfavors and slows down radical rearrangement. This is a potentially very important issue. Cyclopropylcarbinyl radical ring cleavages are known to be sensitive to conformational stereoelectronic effects.[4] A round-trip radical probe which minimizes potential enzyme-imposed adverse conformational stereoelectronic effects should be optimal, or as optimal as can be achieved in practice.

The bicyclo[1.1.1]pentylcarbinyl radical rearrangement may seem fast or slow as a radical probe depending on your perspective. A rate constant of 10^7 s^{-1} (at 25 °C) will seem slow if you judge radical rearrangements by the criteria of how fast a probe has to be to detect a radical intermediate in P_{450} alkane hydroxylation (approximately 10^{10} s^{-1}). However, there are several enzymes that are inactivated by cyclopropyl-containing substrate analogues, presumably via cyclopropylcarbinyl radical rearrangement (10^8 s^{-1}) in many instances.[10] The recent work probing the radical mechanism of isopenicillin N-synthetase illustrates the utility of the relatively "slow" (10^8 s^{-1}) cyclopropylcarbinyl radical (1) as a probe for enzymic radical intermediates.[11] The main point here is that P_{450} may be a special case and that many, if not most, enzymes can be studied using radical probes with rate constants for rearrangement in the range of 10^7–10^8 s^{-1}. For the bicyclo[1.1.1]pentylcarbinyl radical rearrangement the first step with a rate constant of 10^7 s^{-1} is the committed step that will divert radical intermediates away from normal catalysis. The second step with a rate of 10^4 s^{-1} will deliver the radical back into normal catalysis after a brief delay, therefore not affecting the utility of 3 as a probe. In summary, we have reported the ring cleavage–rearrangement of the bicyclo-[1.1.1]pentylcarbinyl radical (3) as the prototypical round-trip radical probe. The concept of round-trip radical probes in general and the bicyclo[1.1.1]pentylcarbinyl radical probe in specific should provide new approaches and new tools to probe for radical intermediates in enzyme reaction mechanisms.

Experimental Section

Materials. Reagents were purchased from Aldrich Chemical Co. unless otherwise specified. *N*-Hydroxy-2(1*H*)-pyridine-2-thione as a 50% aqueous solution of the sodium salt was a gift from Eli Lilly & Co. The neutral compound was obtained by precipitation with concentrated HCl and recrystallization of the *N*-hydroxy-2(1*H*)-pyridine-2-thione from 95% ethanol. Toluene was distilled from CaH_2 and deoxygenated by purging with N_2 prior to use. THF was distilled from sodium benzophenone ketyl under N_2. Methylene chloride was distilled from P_2O_5. Thiophenol was distilled from $CaSO_4$ under N_2 prior to use.

1-[[(Bicyclo[1.1.1]pentylmethyl)carbonyl]oxy]-2(1*H*)-pyridinethione (7).[6a] The radical precursor (7) was prepared from the corresponding bicyclo[1.1.1]pentylacetic acid.[6b] The acid (3.57 mmol, 400 mg), *N*-hydroxy-2(1*H*)-pyridinethione (3.57 mmol, 453 mg), and 4-(dimethylamino)pyridine (0.39 mmol, 44 mg) were dissolved in 30 mL of dry CH_2Cl_2, and the reaction was cooled to 0 °C. Dicyclohexylcarbodiimide (3.93 mmol, 810 mg) was dissolved in 10 mL of CH_2Cl_2, and the resulting solution was added dropwise to the reaction mixture. The reaction vessel was protected with Al foil to shield it from light, and the reaction was stirred overnight. The solvent was evaporated, the residue was dissolved in ethyl acetate, was washed successively with 1 M $KHSO_4$, H_2O, and 5% $NaHCO_3$, and was dried over Na_2SO_4, and the solvent was evaporated to give a yellow-green oil which was recrystallized from benzene–hexanes to give a yellow solid (400 mg, 50% yield), mp 78–80 °C. ^1H NMR ($CDCl_3$): δ 1.98 (s, 6 H), 2.60 (s, 1 H), 2.95 (s, 2 H), 6.68 (dt, 1 H), 7.25 (dt, 1 H), 7.60 (d, 1 H), 7.60 (d, 1 H), 7.75 (dd, 1 H).

Kinetics of 7 in Toluene (Typical Procedure).[3] An 8-in., 10-mm o.d. glass tube was equipped with a stir bar, sealed with a septum, flushed with N_2 through a syringe needle, and protected from light with Al foil. To this was added a stock solution of the precursor 7 in toluene followed by the desired amount of PhSH or *n*-Bu_3SnH and the total volume was adjusted to 1 mL with additional toluene. Concentrations of precursor 7 were typically 0.01–0.02 M in order to ensure pseudo-first-order conditions (hydrogen atom donor in >10-fold excess). The reaction tubes were cooled to −78 °C and sealed under vacuum. The reactions were allowed to warm slowly to room temperature and then placed in a bath and allowed to equilibrate for ca. 2 min at the desired temperature. The Al foil light shield was removed, and the reaction was photolyzed with stirring for 30 min with a 300-W tungsten filament lamp that was placed 10 in. from the reactions. (Reactions were complete in this time as determined by an experiment done in benzene-d_6 in an NMR tube where the precursor peaks in the ^1H NMR were observed to disappear after 30-min irradiation with concomitant appearance of peaks due to the product hydrocarbons.) The reactions were then cooled to −78 °C, opened, and analyzed by gas chromatography (column: 1.8 m × 3.2 mm OD, 10% OV-101 on Chrom W). Products were identified by comparison with authentic samples (vida infra), and yields of hydrocarbons 8 and 6 were typically 90–100% as determined by addition of nonane as an internal standard and employing a predetermined response factor for the methybicyclo[1.1.1]pentane and assuming 1:1 response factors for 8 and 6.

2-(2-Methyl-3-chloropropyl)-1,3-dithiane.[12] Dithiane (5.05 g, 42 mmole) was dissolved in 150 mL of dry THF under an Ar atmosphere. The reaction mixture was cooled to −50 °C and *n*-BuLi (1.57 M, 26.8 mL, 42 mmol) was added dropwise, followed by a solution of 1-bromo-3-chloro-2-methylpropane (7.2 g, 42 mmol) in 10 mL of dry THF. The reaction was allowed to warm to −20 °C and stirred for 12 h. Evaporation of THF from a small sample gave: ^1H NMR: δ 1.06 (d, 3 H), 3.51 (d, 2 H), 4.07 (t, 2 H). The remaining solution was used without further purification or evaporation.

3-Methylcyclobutanone Trimethylene Thioketal. The THF solution from the previous step was cooled to −78 °C, and *n*-BuLi in hexane (1.57 M, 30 mL, 47 mmol) was added dropwise. The reaction was allowed to warm to −20 °C and then was stirred 1 h, warmed to room temperature, and stirred 24 h. Some starting material remained by NMR, and an additional 10 mL of *n*-BuLi was added and the mixture stirred 24 h. The THF was rotary evaporated, and the residue was dissolved in CH_2Cl_2 and washed with H_2O. An emulsion formed, which was broken by the addition of a small amount of acetone. The CH_2Cl_2 phase was dried (Na_2SO_4) and filtered through a short silica gel column, and the solvent was rotary evaporated to give a reddish brown liquid (6.05 g, 82% yield) that was used without further purification or characterization.

1-Methylcyclobutanone. 3-Methylcyclobutanone trimethylene thioketal (6.05 g, 35 mmol) and $HgCl_2$ (25 g, 92 mmol) were dissolved in 200 mL of ethylene glycol along with 5 mL of concd HCl and 25 mL of H_2O. The solution was heated to 130 °C. A slow stream of N_2 was bubbled through the mixture and into a cold trap. An additional 40 mL of H_2O was added, and several mL of distillate were collected. The distillate was extracted with CH_2Cl_2, washed with H_2O and dried (Na_2SO_4). The solvent

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was removed and rotary evaporated and the residue Kugelrohr distilled to give 120 mg (4%), bp 115-120 °C. ^{1}H NMR: δ 1.31 (d, 3 H), 2.53 (m, 1 H), 2.64 (m, 1 H), 3.17 (m, 2 H).

1-Methyl-3-methylenecyclobutane (6). Dimethyl sulfoxide (distilled from CaH_2) was stirred under Ar for 30 min. Methyltriphenylphosphonium iodide (1.45 g, 3.6 mmol) was added and the reaction stirred for 2 h. 3-Methylcyclobutanone (120 mg, 3.6 mmol) was dissolved in 2 mL of DMSO and added to the reaction via syringe. A cannula was connected from the reaction flask to a cold trap cooled in dry ice–acetone. A small amount of liquid was collected, and NMR and GC–MS were taken. ^{1}H NMR:[13] δ 1.13 (d, 3 H), 2.24 (d, 2 H), 2.36 (m, 1 H), 2.80 (m, 2 H), 4.71 (d, 2 H). GC–MS showed M^+ at m/e 82.

Methylbicyclo[1.1.1]pentane (8).[6a] To crude 7 (5.1 mmol, 1.1 g) in a round-bottomed flask that had been flushed with N_2, cooled to −15 °C, and protected from light with Al foil was added thiophenol (10 mL, 0.1 mol) via syringe. The foil was removed and the stirred solution irradiated with a 300-W tungsten filament lamp for 30 min. A cannula was connected from the reaction flask to a cold trap cooled to −78 °C, and the reaction mixture was warmed to 70 °C. A small amount of clear distillate was collected (200 mg, 50%). ^{1}H NMR:[4] δ 1.1 (s, 3 H), 1.66 (s, 6 H), 2.48 (1 H, s). GC–MS showed M^+ peak at m/e 82.

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Registry No. 3, 136379-21-0; 6, 15189-18-1; 7, 136379-22-1; 8, 10555-48-3; 1-bicyclo[1.1.1]pentylacetic acid, 131515-31-6; *N*-hydroxy-2-(1*H*)-pyridinethione, 1121-30-8; 2-(2-methyl-3-chloropropyl)-1,3-dithiane, 53198-70-2; dithiane, 51330-42-8; 1-bromo-3-chloro-2-methylpropane, 6974-77-2; 3-methylcyclobutanone trimethylene thioketal, 136379-23-2; 3-methylcyclobutanone, 1192-08-1; methyltriphenylphosphonium iodide, 2065-66-9.

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Direct Cleavage of Peptides from a Solid Support into Aqueous Buffer. Application in Simultaneous Multiple Peptide Synthesis[1]

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A method of simultaneous multiple peptide synthesis which integrates synthesis, side-chain deprotection, cleavage, and purification so as to afford peptide solutions suitable for immediate biological testing is described. The approach utilizes a novel diketopiperazine-forming cleavable linker **1**. Upon side-chain deprotection, **1** gives **2**, which is stable to a protocol designed to remove contaminants from the support-bound peptide prior to cleavage. Peptide cleavage is then effected by treating **2** with a neutral or near neutral buffer to give peptide **4**, which carries a C-terminal diketopiperazine moiety, in good yield. In this study the glycolamido and 4-(oxymethyl)benzamido esters of **1** have been appraised. The approach is demonstrated in model studies on **7** and **8** and in the preparation and characterization of peptides **17–21**. The general approach allows 10–100-nmol quantities of many hundreds of peptides to be concurrently prepared in a relatively short period of time when used in conjunction with the multipin method of multiple peptide synthesis.

Introduction

Growth in the demand for synthetic peptides has been partly addressed by a range of techniques facilitating rapid peptide synthesis through parallel handiing. Simultaneous multiple peptide synthesis has been performed on resin,[2-5] cellulose[6,7] and grafted polyethylene[8-10] or polypropylene[11] supports. Despite the speed at which peptides can be assembled by a parallel synthesis strategy, the need for individual handling at the side-chain deprotection, cleavage, and purification steps limits the number of peptides that can be conveniently prepared. Several methods of overcoming the postsynthesis bottleneck have been proposed. For example, closely related peptides can be synthesized on the same support and subsequently separated by HPLC.[4] Purification and characterization must be straightforward, however, if this method is to succeed. The use of specialized apparatus designed for multiple peptide cleavage simplifies cleavage and side-chain deprotection[12]

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