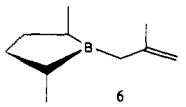
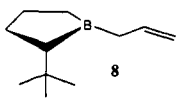
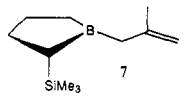
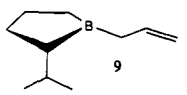
Table II. Allylboration of Isobutyraldehyde with Reagents **6-9** at -78 °C

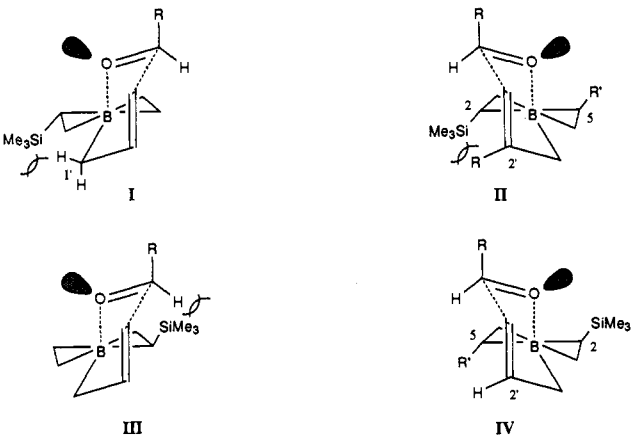
	% ee (% yield)		% ee (% yield)
 **6**	27 (80)	 **8**	72 (95)
 **7**	81 (78)	 **9**	24 (87)

(*S*)-**1**, (*S,S*)-2,5-dimethyl-*B*-allylborolane (**4**), and (*S,S*)-2,5-bis(trimethylsilyl)-*B*-allylborolane (**5**) are summarized together with those recorded for other known chiral reagents (Table I). It is evident that the enantioselectivity of **1** is uniformly high with all achiral aldehydes examined and superior to even **4** and **5**, which were previously thought to be ideally designed for this type of asymmetric induction.[14]

This unexpected result demands some explanations. Since the two faces of the borolane are diastereotopic, approach of the aldehyde from both faces is considered. Thus, provided that (1) the reaction proceeds through a six-membered chelate ring and (2) the aldehyde is coordinated with the larger group anti to the developing B-O bond,[15] four different transition states I-IV are subject to evaluation.

Examination of the absolute configuration of the products obtained employing reagent **1** of established absolute configuration leads immediately to the exclusion of transition states I and III,

Transition States



since these lead to products of configuration opposite to that obtained. A distinction between the two remaining transition states II and IV is drawn by the information provided in Table I, entry 2. The notable reduction in enantioselectivity from 96% ee (with **1**) to 71% ee with **5** may be taken as a manifestation of the steric interaction between C(2′)-H and C(2)-(Me_3Si), indicated in II (R = H), as this repulsion is unavoidable in either II or IV (R = H, R′ = Me_3Si) with **5** but does not exist in IV (R = R′ = H) with **1**.[16] This interpretation is in harmony with the observations revealed in Table II, which show that methyl substitution at C(2′) of **4** (methallylborane **6**)[9] results in significantly reduced enantioselectivity (27% ee) relative to the unsubstituted allyl derivative **4** (85% ee, Table I, entry 2). The 15% ee decrease observed with **7**[9] (2′-methyl substitution on **1**) therefore likely reflects the interaction of the C(2′)-methyl with C(5)-H in IV (R = Me, R′ = H) rather than that of the C(2′)-methyl with C(2)-(Me_3Si) in II (R = Me, R′ = H). Thus, IV appears to be energetically favored relative to II.

The asymmetric induction brought about by **1** is largely of steric origin; any stereoelectronic component, if active, is not evident. The enhanced enantioselectivity exhibited by **1** relative to **8** and **9** (Table II) is due to the greater effective "reach" of trimethylsilyl versus *tert*-butyl and isopropyl, respectively, a consequence of the increased C-Si bond length present in **1**. Comparison of transition state IV with transition state I (which collapses to the minor enantiomer) suggests that the Me_3Si group of **1** senses the difference between C(1′)-H_2 and the (uncoordinated) syn lone pair of the aldehydic oxygen. Indeed, aldol reactions with *B*-(vinyloxy)-2-(trimethylsilyl)borolanes (replacing the C(1′)-H_2 by oxygen) proceed with virtually no enantioselection.[17]

Acknowledgment. We thank Dr. John C. Dewan for the crystallographic analysis of (+)-**3** and the National Institutes of Health (GM 35879) for financial support.

Supplementary Material Available: Detailed experimental procedures and tables of crystallographic data (positional parameters, *U* values, and bond distances and bond angles) for (+)-**3** (33 pages). Ordering information is given on any current masthead page.

(14) (a) Short, R. P.; Masamune, S. *Tetrahedron Lett.* **1987**, *28*, 2841. Garcia, J.; Kim, B. M.; Masamune, S. *J. Org. Chem.* **1987**, *52*, 4831.
(15) Reetz, M. T.; Hullmann, M.; Massa, W.; Berger, S.; Rademacher, P.; Heymanns, P. *J. Am. Chem. Soc.* **1986**, *108*, 2405.
(16) With reagent **4**, this interaction is between C(2′)-H and C(2)-Me. The smaller steric demand of Me relative to $SiMe_3$ is reflected in the higher enantioselectivity of **4** vs **5**.
(17) The boron enolate derived from 3-(3-ethyl)pentyl ethanethioate (ref 2b) and (*S*)-2-(trimethylsilyl)-*B*-chloroborolane was evaluated. Cho, J.-H.; Masamune, S., unpublished results.

Characterization of the Equilibrating Forms of the Aldehydic Abasic Site in Duplex DNA by ^{17}O NMR

Joyce A. Wilde and Philip H. Bolton*

Department of Chemistry, Wesleyan University Middletown, Connecticut 06457

Abhijit Mazumder, Muthiah Manoharan, and John A. Gerlt*

Department of Chemistry and Biochemistry University of Maryland College Park, Maryland 20742

Received October 5, 1988

The enzymatic repair of chemical and physical damage to the bases in DNA is frequently initiated by hydrolysis of the N-glycosidic bond to the damaged base to yield an abasic site.[1,2] For example, the ubiquitous uracil-DNA glycosylase removes the uracil formed in DNA by spontaneous hydrolysis of the 4-amino group of cytosine, and UV endonuclease V from bacteriophage T_4 hydrolyzes one of the glycosidic bonds in a pyrimidine photodimer. The resulting abasic site is a mixture of aldehyde, hydrate, and cyclic hemiacetals. The abasic sites so generated are removed enzymatically from the DNA duplex by cleavage of the phosphodiester backbone at both sides of the abasic site followed by insertion of the proper nucleotide unit in the gap so produced.

While aldehydic abasic sites are important intermediates in DNA repair, little detailed information is available about their structure and chemical reactivity.[3,4] We recently used ^{13}C NMR to study defined DNA duplexes containing an abasic site specifically labeled with ^{13}C in the aldehydic carbon and concluded that the mixture of cyclic hemiacetals is the predominant form of the abasic site in the duplex, that the conformation of a duplex

(1) Lindahl, T. *Annu. Rev. Biochem.* **1982**, *51*, 61-87.
(2) Friedberg, E. C. *DNA Repair*; W. H. Freeman: New York, 1985.
(3) Jones, A. R.; Mian, A. M.; Walker, R. T. *J. Chem. Soc.* **1968**, 2042-2044.
(4) Lindahl, T.; Anderssen, A. *Biochemistry* **1972**, 3618-3623.

0002-7863/89/1511-1894$01.50/0

containing an abasic site depends upon the identity of the base opposite the abasic site, and that the presence of an abasic site in a duplex is less destabilizing than a base mismatch.[5] We subsequently utilized a duplex containing a ^{13}C labeled abasic site to demonstrate that the cleavage of the phosphodiester bond on the 3'-side of the abasic site catalyzed by UV endonuclease V proceeds by a β-elimination mechanism.[6] This chemistry presumably occurs via the minor open chain aldehyde form of the abasic site.

A complete understanding of the chemistry and reactivity of aldehydic abasic sites requires knowledge of the relative amounts of the aldehyde, hydrate, and cyclic hemiacetals. We attempted to obtain this information by ^{13}C NMR studies of duplexes containing abasic sites but failed since the natural abundance signals from the base carbons are in the same chemical shift region and of the same intensity, approximately 1%, that might be expected for the aldehyde.

In contrast to ^{13}C, we hypothesized that the natural abundance of ^{17}O is sufficiently low (0.04%) to eliminate signals associated with the various phosphate, sugar, and base oxygens of a heteroduplex. In addition, the crucial aldehydic and hemiacetal oxygens can be easily selectively labeled with ^{17}O either by removing uracil from a precursor with uracil-DNA glycosylase in $H_2{}^{17}O$ or, more simply, by chemical exchange with $H_2{}^{17}O$. The chemical shifts of aldehyde oxygens occur between 500 and 600 ppm downfield of water, and the chemical shifts of alcohol oxygens are typically between 0 and 100 ppm downfield of water.[7,8]

Two labeled samples of the DNA heteroduplex d(GCGDGCG) paired with d(CGCACGC), referred to as AD_I where D represents the abasic site, have been prepared as follows. The first sample was labeled simply by dissolving the unlabeled duplex[5] in a buffer prepared in 40% enriched $H_2{}^{17}O$. The second sample was labeled by removing uracil from d(GCGUGCG) with uracil-DNA glycosylase[5] in $H_2{}^{17}O$ and annealing to the complementary strand with the $H_2{}^{17}O$ retained as solvent. ^{31}P NMR spectra of these samples revealed the absence of any resonance associated with a phosphomonoester, thereby confirming the integrity of the phosphodiester backbone at the abasic site. The ^{17}O NMR results obtained on the samples prepared by both methods were identical.

Typical ^{17}O NMR spectra for a 2 mM sample of labeled AD_I are shown in Figure 1. The signal near 100 ppm can be assigned to the hydroxyl oxygens of both cyclic hemiacetals, and the signal near 520 ppm can be assigned to the carbonyl oxygen of the aldehyde.[9] No signal associated with the hydrate could be resolved, although the hydroxylic signal at 100 ppm may also include its resonance. The intensity of the resonance associated with the aldehyde oxygen is approximately 1% of that of the resonance associated with the hydroxylic oxygens.[14] The necessary dynamic range for observing approximately 0.01 mM aldehyde in the presence of 20 M ^{17}O in the solvent was achieved by appropriate placement of the transmitter so that the solvent signal would be reduced in intensity by folding (filtering).

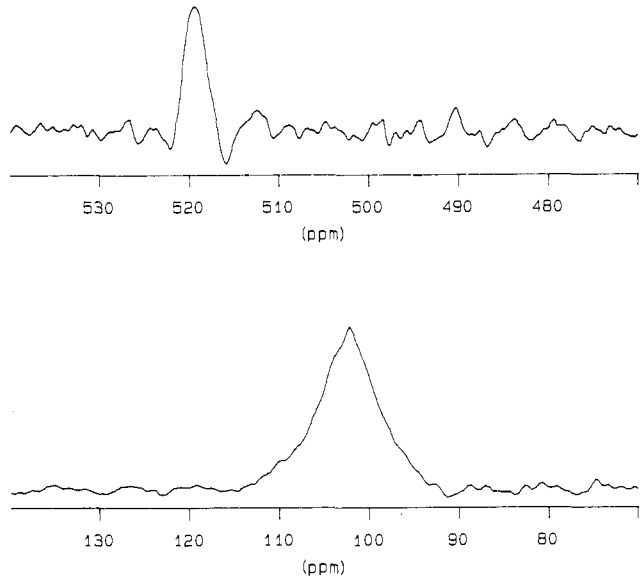


Figure 1. ^{17}O NMR spectra at 54.2 MHz of the labeled heteroduplex AD_I containing the aldehydic abasic site. The spectrum on the bottom is of the hydroxylic oxygens and that on the top is of the aldehyde oxygen. For the lower spectrum the acquisition time was 100 ms with an 80-μs delay after a 25-μs 90° pulse; the spectral width was 22000 Hz. The spectrum was acquired at 20 °C and is the sum of 16400 transients. For the upper spectrum the acquisition time was 150 ms with an 80-μs delay after a 25-μs 90° pulse; the spectral width was 16000 Hz. This base line corrected spectrum was acquired at 20 °C and is the sum of 250000 transients (total acquisition time 10.5 h). For both spectra line broadening of 50 Hz was applied. Dynamic range problems for the aldehyde region of the spectrum were overcome by folding in the $H_2{}^{17}O$ resonance once; this folding reduces the intensity of the ^{17}O signal from the solvent by approximately 250-fold and allows the aldehyde signal to be digitized. The line shape distortion of the signals can be attributed to acoustic ringing of the NMR probe.[7] The ratio of the vertical scales in the top and bottom spectra is 30. Due to base line corrections and line shape distortion the ratio of the integrated intensities of the aldehyde and hemiacetal resonances cannot be determined more accurately than within a factor of two. Both spectra were obtained with a Varian VXR-400 NMR spectrometer.

The distribution among aldehyde, hydrate, and hemiacetal forms has been determined for a number of aldoses and aldose phosphates by Barker and co-workers by using ^{13}C labeled samples; the aldehyde content was in the range of 0.1–1%.[15] Thus, the aldehyde content of an abasic site in a DNA duplex is not strongly influenced by the geometry of the duplex, although we had considered that the structure of the duplex could have significantly reduced the aldehyde content by spatially approximating the aldehyde and 4-hydroxyl group of the abasic site. Since the aldehyde content of the abasic site in this heteroduplex is present at a "normal" level, the significantly lower reactivity of abasic sites in double-stranded DNA relative to single-stranded DNA[16] is likely to be due to restricted access of base to the α-protons of the reactive aldehyde in double-stranded DNA. The aldehyde

(5) Manoharan, M.; Ransom, S. C.; Mazumder, A.; Gerlt, J. A.; Wilde, J. A.; Withka, J. A.; Bolton, P. H. *J. Am. Chem. Soc.* **1988**, *110*, 1620–1622.
(6) Manoharan, M.; Mazumder, A.; Ransom, S. C.; Gerlt, J. A.; Bolton, P. H. *J. Am. Chem. Soc.* **1988**, *110*, 2690–2691.
(7) Gerothanassis, I. P.; Lauterwein, J. *J. Magn. Reson.* **1982**, *48*, 431–446.
(8) Klemperer, W. G. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 246–263.
(9) The chemical shift of the aldehyde oxygen is significantly more upfield than those observed for saturated[10] or α,β-unsaturated[11] aldehydes. We hypothesize that this chemical shift is the result of hydrogen bonding with H_2O within the abasic site in the duplex.[7,8,12,13] This chemical shift confirms both the duplex structure and the integrity of the phosphodiester backbone at the abasic site. The resonance of the hemiacetal oxygens is somewhat more downfield than that usually observed for alcohol oxygens, but this may be explained by hydrogen-bonding effects which can cause downfield shifts of approximately 20 ppm;[13] the upfield shift of this resonance by approximately 5 ppm upon thermal denaturation is consistent with such effects. That this resonance is associated with the hemiacetal oxygens is also supported by our observation that these oxygens exchange rapidly with solvent at 20°. Finally, both assignments are supported by the ^{17}O NMR chemical shifts of the aldehyde and hemiacetal oxygens of deoxyribose-5-phosphate, 550 and 65 ppm, respectively, at pH 5.
(10) Christ, H. A.; Diehl, P.; Schneider, H. R.; Dahn, H. *Helv. Chim. Acta* **1961**, *44*, 865–880.
(11) Delseth, D.; Nguyễn, T. T.-T.; Kintzinger, J.-P. *Helv. Chim. Acta* **1980**, *63*, 498–503.
(12) Canet, D.; Goulon-Ginet, C.; Marchal, J. P. *J. Magn. Reson.* **1976**, *22*, 537–542.
(13) Reuben, J. *J. Am. Chem. Soc.* **1969**, *91*, 5725–5729.
(14) Due to the base line corrections that were made, the accuracy of this estimate of the relative intensities of the aldehyde and hemiacetal signals is only accurate to within a factor of two. This uncertainty does not detract from our ability to observe this signal and the importance of its intensity. To check our method, the aldehyde content of deoxyribose-5-phosphate has been determined by comparing the intensities of the aldehyde and hemiacetal ^1H NMR resonances and the intensities of the aldehyde and hemiacetal ^{17}O NMR resonances; both methods yield the same result, namely 1%.
(15) Pierce, J.; Serianni, A. S.; Barker, R. *J. Am. Chem. Soc.* **1985**, *107*, 2448–2456.
(16) Lafleur, M. V. M.; Woldhuis, J.; Loman, H. *Nucl. Acids Res.* **1981**, *9*, 6591–6599.

content we have determined should be useful in understanding the relative reactivities of aldehydic and deoxyribonolactone abasic sites to strand cleavage.

Finally, since ^{17}O NMR chemical shifts are sensitive to hydrogen bonding effects,[7,8,12,13] we anticipate that ^{17}O NMR spectroscopy can also be used as a probe of the accessibility of the abasic site to solvent molecules.

Acknowledgment. This research was supported by NIH GM-34572 to J.A.G., NIH GM-34573 to J.A.G. and P.H.B., and by grants from the State of Connecticut, Department of Higher Education, and Bristol-Myers to P.H.B.

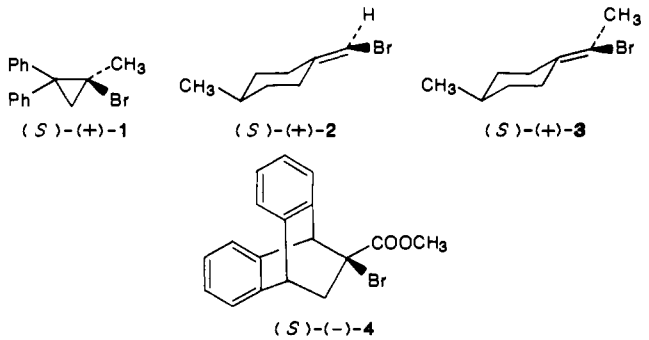
Mechanism of Grignard Reagent Formation. The Surface Nature of the Reaction[1]

H. M. Walborsky* and Janusz Rachon[2]

Dittmer Laboratory of Chemistry
Florida State University
Tallahassee, Florida 32306
Received August 12, 1988

There is a general agreement that the formation of Grignard reagents involve the intermediacy of free radicals.[3-5] The question of whether these radicals are adsorbed on the magnesium surface or diffuse freely in solution has recently been raised by a group of workers[6] who analyze a mechanism ("D-Model") in which the radicals "diffuse freely in solution at all times". The data for the formation of Grignard reagents from *primary alkyl halides* was shown to be consistent with this D model, but herein we show that extrapolation to other substrates may be misleading.

Twenty-seven years ago,[4] it was demonstrated that the reaction of chiral (*S*)-(+)-1-bromo-1-methyl-2,2-diphenylcyclopropane (**1**)



with magnesium powder resulted in the formation of a chiral Grignard reagent which had an optical purity of ca. 12%. It was suggested at the time and later substantiated[7] that the partial racemization observed occurred at the Grignard formation step due to the formation of the 1-methyl-2,2-diphenylcyclopropyl σ radical intermediate. When this same radical is generated *in solution* from a variety of chiral precursors, the resultant products are always racemic,[8] an expected result in view of K. U. Ingold's[9] finding that the rate of inversion of the 1-methylcyclopropyl σ radical is on the order of 10^{11} s^{-1} at 71 °C. Only the surface nature of the Grignard formation reaction provides a reasonable explanation for this chirality and for the observation that (*S*)-(+)-4-methylcyclohexylidenebromomethane (**2**) forms a Grignard reagent which is 47% optically pure.[10] Here again, the vinyl σ radical intermediate is reported[11] to invert its configuration between 10^8–10^{10} s^{-1} at −170 °C.

We now wish to report further results of our study designed to help answer the question of whether or not radicals formed during Grignard formation "diffuse freely in solution at all times". It was decided that methanol-*O-d* would be the solvent of choice since, if the radicals left the surface of the magnesium, then the reactive σ radicals formed from the chiral bromides **1**, **3**, and **4** would react with the solvent to abstract a hydrogen atom from the methyl group[12] to yield R–H. If, on the other hand, it was the Grignard reagent that left the surface then it would be quenched by the methanol-*O-d* to give deuterated product R–D. In order to keep the magnesium surface clean and free of magnesium methoxide a continuous stream of carbon dioxide was passed through the reaction mixture[13] to form methyl magnesium carbonate which is soluble in methanol. The reaction of magnesium with methanol also produces D_2 which could perhaps reduce the alkyl halide. To check this possibility a reaction was carried out so that a stream of hydrogen as well as the CO_2 was passed through the reaction mixture. The product obtained did not contain any R–H. Finally, consideration was given to the possibility that hydridomagnesium methoxide might be a transient reducing agent.[14] To this end we prepared[15] MgH_2 and to it added a solution of **1** dissolved in methanol. The starting material **1** was recovered unchanged in 98% yield. Thus, we feel confident that we are observing the usual Grignard formation reaction.

$$Mg + CH_3OD \rightarrow CH_3OMgD \quad (1)$$

$$CH_3OMg\text{-}D + CH_3OD \rightarrow (CH_3O)_2Mg + D_2 \quad (2)$$

$$(CH_3O)_2Mg + 2CO_2 \rightarrow (CH_3OCO_2)_2Mg \quad (3)$$

As one can see from Table I the yields of R–D are almost quantitative, and the optical purities decrease with decreasing s-character of the orbital involved, **3** > **1** > **4**. Of significance is the observation that **4**, which would give rise to a planar π radical delocalized by an adjacent carbomethoxy group, still yields a product with 11% optical purity. This speaks strongly for an adsorbed radical on the surface of magnesium as does the fact that the rapidly inverting σ radicals 1 and 3 also retain a large

Table I. Reaction of Magnesium in Methanol-*O-d* with R–Br To Yield R–D

R–Br	yield, % R–H + R–D	yield, % R–D	optical purity, %	retentn of configurtn, %
1	87	100[b]	23	72
3[a]	70	93[b,c]	60	80
4[a]	94	95[d]	11	44[e]

[a] The syntheses of these compounds will be reported elsewhere. [b] Determined by using mass spectroscopic analysis, by Prof. Roy King, University of Florida. [c] Minimum value. [d] Determined by NMR, minimum value. [e] Overall inversion as expected, see: Annino, R.; Erickson, R. E.; Michalovic, J.; McKay, B. *J. Am. Chem. Soc.* **1966**, *88*, 4424.

(1) This work was supported by a grant (CHE 8503227) from the National Science Foundation. A NATO Travel Grant (517/87) to one of us (H.M.W.) is also appreciated.

(2) Visiting Professor from the Institute of Organic Chemistry, Technical University, Gdansk, Poland.

(3) Gomberg, M.; Bachmann, W. E. *J. Am. Chem. Soc.* **1927**, *49*, 236. Kharasch, M. S.; Reinmuth, C. *Grignard Reactions of Nonmetallic Substances*; Prentice-Hall, Inc.; 1954. Bryce-Smith, D.; Cox, G. F. *J. Chem. Soc.* **1958**, 1050. Anteunis, M.; Van Schook, J. *Bull. Soc. Chim. Belg.* **1963**, *72*, 787. Ruchhardt, C.; Trautwein, H. *Chem. Ber.* **1962**, *95*, 1197. Grovenstein, E.; Cottingham, A. B.; Gelbaum, L. T. *J. Org. Chem.* **1978**, *43*, 3332.

(4) Walborsky, H. M.; Young, A. E. *J. Am. Chem. Soc.* **1961**, *83*, 2595. Walborsky, H. M. *Record Chem. Prog.* **1962**, *23*, 75.

(5) Grotveld, H. H.; Blomberg, C.; Bickelhaupt, F. *Tetrahedron Lett.* **1971**, 1999.

(6) Garst, J. F.; Deutsch, J. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1986**, *108*, 2490.

(7) Walborsky, H. M.; Young, A. E. *J. Am. Chem. Soc.* **1964**, *86*, 3288. Walborsky, H. M.; Aronoff, M. S. *J. Organomet. Chem.* **1973**, *51*, 31.

(8) For a review of the cyclopropyl radical, see: Walborsky, H. M. *Tetrahedron* **1981**, *37*, 1626. Boche, G.; Walborsky, H. M. *Chemistry of the Cyclopropyl Group*; Rappoport, Z., Ed.; John Wiley & Sons: New York, 1987; Part 1, Chapter 12, pp 702–808.

(9) Johnson, L. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1986**, *108*, 2343.

(10) Walborsky, H. M.; Banks, R. B. *Bull. Soc. Chim. Belg.* **1980**, *84*, 849 and references cited therein.

(11) Fessenden, R. W.; Schuler, R. H. *J. Chem. Phys.* **1963**, *39*, 2147.

(12) (a) Benson, S. W. *J. Chem. Ed.* **1965**, *42*, 502. (b) Packer, J. E.; House, D. B.; Rasburn, E. J. *J. Chem. Soc.* **1971**, *13*, 1574.

(13) No carboxylic acids are formed under these conditions.

(14) Firestone, R. A. *Tetrahedron Lett.* **1967**, *27*, 2629.

(15) Ashby, E. C.; Kovar, R. A.; Kawakami, K. *Inorg. Chem.* **1970**, *9*, 317.

0002-7863/89/1511-1896$01.50/0