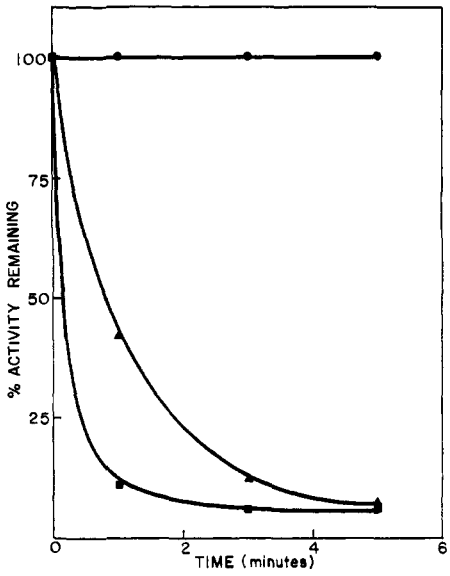

Figure 1. Irreversible inhibition of monoamine oxidase by 3-bromoallylamine: (●) control, (▲) 1 m*M* 3-bromoallylamine, (■) 3 m*M* 3-bromoallylamine. Seven units of enzyme (specific activity = 30 units/mg) was incubated in 0.2 *M* phosphate buffer, pH 7.5, with either 3 or 1 m*M* freshly prepared 3-bromoallylamine along with a control. The remaining activity with time was measured by diluting the enzyme 50-fold in 3.3 m*M* benzylamine and measuring the increase in OD at 250 nm (benzaldehyde λ_{max} 250 nm).[6] The activity of the inhibited enzymes could not be restored in the slightest by continued dialysis, attesting to their being irreversibly inhibited.

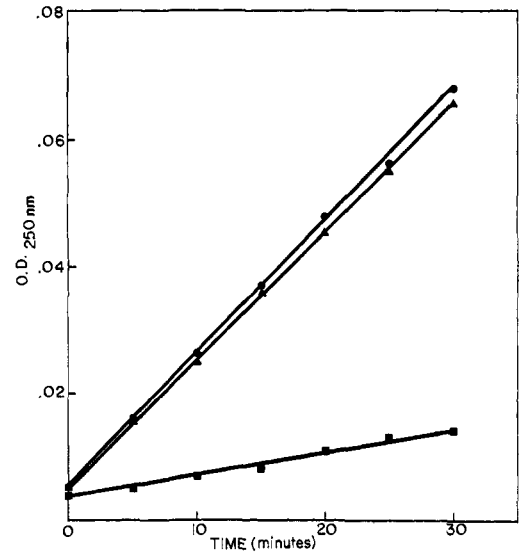

Figure 2. Protection against irreversible inhibition by the substrate: (●) control, (▲) 10 m*M* benzylamine + 0.2 m*M* 3-bromoallylamine, (■) 0.2 m*M* 3-bromoallylamine. Five units of the enzyme were each preincubated with the above solutions for 5 min. Aliquots were then removed and assayed by the standard benzylamine assay.

Indeed, 3-bromoallylamine is a potent irreversible inhibitor of the enzyme as will be demonstrated here.

Incubation of rat liver mitochondrial monoamine oxidase with 3-bromoallylamine led to its rapid and irreversible inhibition as demonstrated in Figure 1.[6,7] If the reagent is active-site directed then one must demonstrate protection against inactivation by the normal substrate. This is shown in Figure 2. A demonstration of the precise mode of irreversible inhibition awaits isolation and characterization of the labeled active-site peptide fragment. Interestingly enough flavine-linked monoamine oxidase can be irreversibly inactivated by the drug pargyline (*N*-methyl-*N*-2-propynylbenzylamine) and the flavine-linked, bacterial lactate dehydrogenase by 2-hydroxy-3-butynoic acid.[8,9] It is highly likely that an acetylene to allene conversion mediates the actual inhibition.

In order to achieve the kind of irreversible inhibition reported here, certain prerequisites must be met. First, the enzyme must be capable of carrying out the conversion of the chemically unreactive species to the reactive one. Secondly, the reactive moiety must be generated within bonding distance of an appropriate residue. Finally, the reactive moiety must remain at the active site for a sufficient amount of time to allow for a chemical reaction. All of these criteria are met in the examples described here. The last criterion, though not as obvious as the first two, is crucial to the successful design of these inhibitors. Since the rate of enzyme–substrate complex dissociation will normally proceed more rapidly than nonphysiological covalent bond formation, these inhibitors will be more of an exception than a rule unless steps are taken to ensure a slow dissociation rate. Of course, when the enzymatic mechanism is based on covalent catalysis this task is simplified.

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(6) Rat liver mitochondria were prepared by the method of W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, **183**, 123 (1950). The activity of the enzyme was measured by the standard benzylamine assay: C. W. Tabor, H. Tabor, and S. M. Rosenthal, *ibid.*, **208**, 645 (1954).

(7) (a) 3-Bromoallylamine was prepared after A. Clavier, *Bull. Soc. Chim. Fr.*, 646 (1954). A mixture of the cis and trans isomers has been used in these experiments. We are currently separating the two isomers to test if both are inhibitors. (b) We have found that 3-chloroallylamine will also induce the irreversible inhibition of the enzyme. Pure *cis*-and *trans*-3-chloroallylamines can easily be prepared and only the cis compound is an irreversible inhibitor of the enzyme. Although the trans isomer can bind to the enzyme it cannot irreversibly inhibit it, and indeed is not a substrate for the enzyme. These observations attest to the high order of specificity implicit in the mechanism of action of these inhibitors.

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Homolytic Reactions of Grignard Reagents and Organomagnesium Compounds by Electron Spin Resonance

Sir:

Most reactions of Grignard reagents are considered to take place by essentially ionic mechanisms, although recent chemical evidence indicates the possibility of free-radical and electron-transfer processes occurring in some cases.[1,2] We wish to describe the identifica-

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Table I. Free Radicals from Organomagnesium and Grignard Reagents in Diethyl Ether

Source	Radical Obsd	Temp, °C	⟨g⟩	a_α^H, G	a_β^H, G
$(CH_3)_2Mg$	$\cdot CH_2MgCH_3$	−106	2.00279	18.54	
	$\cdot CH_3$		2.00262	22.75	
CH_3MgCl	$\cdot CH_2MgCl$	−100	2.00286	18.62	
	$\cdot CH_3$		2.00266	22.80	
$(CH_3CH_2)_2Mg$	$CH_3\dot{C}HMgC_2H_5$	−99	2.00293	18.24	24.35
	$CH_3CH_2\cdot$		2.00278	22.28	27.00
CH_3CH_2MgCl	$CH_3\dot{C}HMgCl$	−103	2.00296	17.94	24.10
	$CH_3CH_2\cdot$		2.00279	22.22	27.10
$[(CH_3)_2CH]_2Mg$	$(CH_3)_2\dot{C}MgC_3H_7$	−116	2.00280		21.76
	$(CH_3)_2CH\cdot$		2.00267	22.1	24.67
$(CH_3)_2CHMgCl$	$(CH_3)_2\dot{C}MgCl$	−99	2.00287		21.79
	$(CH_3)_2CH\cdot$		2.00273	21.97	24.68
$(CH_3CH_2CH_2)_2Mg$	$CH_3CH_2\dot{C}HMgC_3H_7$	−108	2.00303	18.12	28.70
	$CH_3CH_2CH_2\cdot$		2.00287	22.13	31.17
CH_3CH_2MgF[a]	$CH_3\dot{C}HMgF$[b]	−92	2.00293	18.43	24.02

[a] In THF. [b] No fluorine hfs resolved.

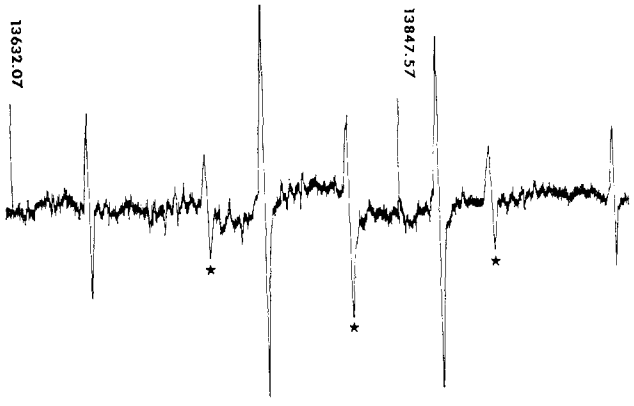

Figure 1. Esr spectrum of the methyl and magnesiomethyl (starred) radicals obtained from the reaction of dimethylmagnesium and *tert*-butoxy radicals at −103° in diethyl ether solution. Proton nmr field markers are in kilohertz.

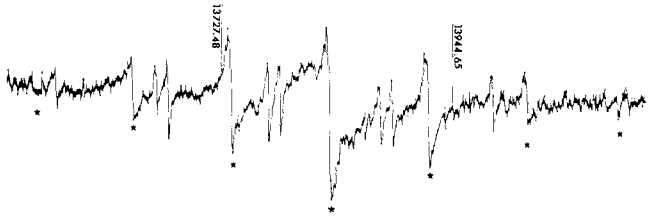

Figure 2. Esr spectrum of the isopropyl radical and α-magnesioisopropyl radical (starred) from diisopropylmagnesium and the *tert*-butoxy radical in diethyl ether at −115°.

$$R_2CHMgX + t\text{-}BuO\cdot \longrightarrow \begin{cases} R_2\dot{C}H + t\text{-}BuOMgX & (1)^4 \\ \quad \text{III} & \\ R_2\dot{C}MgX + t\text{-}BuOH & (2) \\ \quad \text{IV} & \end{cases}$$

tion by esr of paramagnetic species derived from organomagnesium and Grignard reagents.

Dimethylmagnesium (halide-free from dimethylmercury) in ether solution reacted with photochemically generated *tert*-butoxy radicals to afford the spectrum shown in Figure 1, which consists of the superposition of the spectra of methyl radical I and magnesiomethyl radical II.[3] A similar spectrum was generated from CH_3MgCl, but the hyperfine splitting by Cl-35 or -37 was not evident in the spectrum of the magnesium-containing radical.

Ethyl, *n*-propyl, and isopropyl analogs of both dialkylmagnesium and alkylmagnesium chloride were also treated under the same conditions. In each instance, the esr spectrum consisted of the superposition of the individual spectra of two species with different *g* factors. One component was clearly that of the well-characterized alkyl radical III derived by fission of the alkyl–magnesium bond (eq 1). The other species was identified as the 1-magnesioalkyl radical IV by its hyperfine splitting pattern, which is consistent with the removal of the α hydrogen from the alkylmagnesium precursor. Thus, the spectrum of IV derived from diisopropylmagnesium consisted of a septet with the correct binomial intensity ratio (Figure 2), and that from diethylmagnesium was a quartet of doublets. In all of these studies, the spectrum disappeared on shuttering the light and was not observed in the absence of peroxide. The esr parameters for both III and IV obtained under these conditions are listed in Table I.

The S_H2 displacement of alkyl radicals in eq 1 may occur synchronously or by a stepwise addition–elimination sequence.[5] As yet we have been unable to observe the spectrum of any paramagnetic species attributable to a magnesium-centered radical[6] expected to be an intermediate in the stepwise process. We conclude that if an adduct is formed, it must have a fleeting existence even at −130°.

α-Magnesioalkyl radicals IV have not been reported in the free-radical chemistry of Grignard reagents. Their presence as ubiquitous species in the esr studies suggests, however, that they may play an important role, since the good S/N ratios in the spectra indicate

(3) Samples were prepared by mixing *in vacuo* the organomagnesium compound and di-*tert*-butyl peroxide at −78°. The spectra of III and IV appear immediately on irradiation.

(4) X in eq 1 and 2 is halide and alkyl when Grignard and dialkylmagnesium compounds, respectively, are used. The possibility of exchange with *tert*-butoxide, however, cannot be ruled out, but is unlikely (see ref 3).

(5) A. G. Davies and B. P. Roberts, *Accounts Chem. Res.*, 5, 387 (1972); J. K. Kochi and P. J. Krusic, *J. Amer. Chem. Soc.*, 91, 3942, 3944 (1969).

(6) (a) However, an unassigned spectrum with ⟨g⟩ = 2.00294 consisting of a triplet (1.76 G) of triplets (33.60 G) was also observed from ethylmagnesium fluoride. (b) Spectra of III and IV can also be obtained from alkylmagnesium bromides, but the lines were generally broader.

Table II. Comparative Electron Spin Resonance Parameters for α-Magnesioalkyl Radicals and Alkyl Radicals

Radical	Temp, °C	⟨g⟩	a_α^H, G	a_β^H, G	ρ_α
$CH_3\dot{C}HMgCH_2CH_3$	−99	2.00293	18.24	24.35	0.83
$CH_3\dot{C}H_2$	−99	2.00278	22.28	27.00	0.92
$CH_3\dot{C}HCH_3$	−99	2.00273	21.97	24.68	0.84
$CH_3\dot{C}HOCH_2CH_3$	−47	2.00330	13.93	21.72	0.74

that IV is present in relatively high concentrations.[7] The steady-state concentration of IV relative to III depends on the organomagnesium compound and is generally higher from RMgCl than from R_2Mg and in diethyl ether compared to tetrahydrofuran. Part of the difference is related to the variation in the degree of association of organomagnesium compounds with structure and the solvent.[8] It is especially noteworthy that CH_3CH_2MgF, which is *strongly* dimeric in solution,[9] affords only IV and no III.

The hydrogen splitting in the magnesiomethyl radical II is significantly smaller than that in the methyl radical I. Spin withdrawal from carbon by the magnesium atom is probably similar to that of the lithiomethyl radical described earlier.[10] An estimate of the spin density (ρ_α) on the α carbon can be obtained from the Fessenden–Fischer relationship[11] in eq 3 for the series

$$a_{CH_3} = Q^{CH_3}\rho_\alpha = 29.30\rho_\alpha \qquad (3)$$

of radicals in Table II. Furthermore, the small difference in the g factors between IV and III is associated with the rather small spin–orbit coupling constant for magnesium.[12]

Acknowledgment. We wish to thank the National Science Foundation for financial support of this work, Dr. E. Ashby for a discussion of the preparation of ethylmagnesium fluoride, and NATO (Paris) for a fellowship (J.-P. B.).

(7) (a) Strictly speaking, its importance in the reaction will depend on its lifetime relative to that of alkyl radicals. (b) It should be noted that *tert*-butoxy radicals are efficient in hydrogen abstraction reactions. (c) Furthermore, the spectra of III and IV appear under conditions in which the spectra of the radicals derived from the solvent (diethyl ether or THF) were not observed (*e.g.*, see Figures 1 and 2), despite the favorable reactivities of ethers as hydrogen donors. Well-resolved spectra of α-oxyalkyl radicals were readily obtained under the same conditions in the absence of organomagnesium compounds (J. K. Kochi and P. J. Krusic, *Chem. Soc., Spec. Pub.*, No. **24**, 147 (1970).

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(11) H. Fischer, *Z. Naturforsch. A*, **20**, 428 (1965); R. H. Fessenden and R. W. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).

(12) Mg^0, $\xi_{np} = 40\ cm^{-1}$; Mg^I, $\xi_{np} = 61\ cm^{-1}$. Calculations based on the Lande interval rule; atomic spectral data from C. E. Moore, *Nat. Bur. Stand. (U. S.) Circ.*, **2**, No. 467 (1952).

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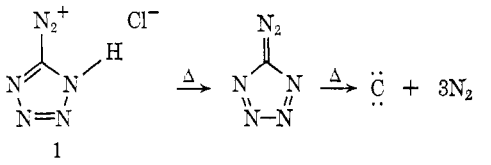
The Reaction of Chemically Generated Carbon Atoms with Carbon Monoxide

Sir:

We recently reported the formation of atomic carbon in the thermal decomposition of 5-tetrazoyldiazonium chloride (**1**).[1] The reactions of atomic carbon have



1

been studied by such methods as generation of carbon atoms by nuclear reaction[2] and production of the atoms in a carbon arc;[3] the thermolysis reported here offers an alternative pathway for the production of carbon atoms. We wish to report additional reactions which provide further evidence for the formation of carbon atoms in the decomposition of **1**.

The reactions of carbon atoms are conveniently studied by coating **1** on the walls of a flask and thermally (120°) decomposing it in the presence of a gaseous reactant. When **1** is allowed to decompose in an atmosphere of carbon monoxide, a major product is carbon suboxide (**2**). The most plausible pathway for the formation of carbon suboxide is initial reaction of atomic carbon with carbon monoxide to give C_2O. The C_2O subsequently reacts with an additional molecule of carbon monoxide to generate carbon suboxide (eq 1).

$$C + CO \longrightarrow :C{=}C{=}O \qquad \Delta H = -62\ kcal$$
$$:C{=}C{=}O + CO \longrightarrow O{=}C{=}C{=}C{=}O \qquad (1)$$
$$\Delta H = -108\ kcal$$

Thermochemical considerations[4] indicate that, irrespective of the spin state of the various intermediates, both of the reactions in eq 1 are exothermic. The heats of reaction are for $C(^1D)$ reacting to produce C_2O ($^1\Sigma$) and ground-state carbon suboxide.

Nucleogenic carbon-11 atoms have been shown to react with carbon monoxide to give small yields of carbon suboxide.[5] The major product in this oxygen-scavenged system was ^{11}CO. Jacox and coworkers,[6] generating carbon atoms by photolysis of cyanogen azide in an argon matrix at 14°K, have observed a reaction with carbon monoxide to produce both C_2O and carbon suboxide. The reactive species in this study was thought to be $C(^3P)$. The vacuum-ultraviolet photolysis of carbon monoxide, in which $C(^1D)$ is produced, gives carbon suboxide as one of the products.[7] Husain and Kirsh[8] have reported flash photolysis studies of carbon suboxide in which the absorptions of both $C(^1D)$ and $C(^3P)$ are quenched by added carbon monoxide. The rate constant for the reaction of $C(^1D)$ with carbon monoxide, obtained by Husain and Kirsh, is far greater than that for $C(^3P)$ with carbon monoxide. Thus, there appears to be ample analogy for the reaction of both singlet and triplet carbon atoms with carbon monoxide to produce carbon suboxide.

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(3) P. S. Skell, J. J. Havel and M. J. McGlinchey, *Accounts Chem. Res.*, **6**, 97 (1973).
(4) E. Tschuikow-Roux and S. Kodama, *J. Chem. Phys.*, **50**, 5297 (1969).
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