Table I. Oxidation of [1,2-³H]Cholesterol by Rat Liver Subcellular Fractions

Sterol product	Mitochondrial fraction[b]	Microsomal fraction[c] With linoleate[d]	Microsomal fraction[c] With linoleate hydroperoxides[e]		Soluble fraction[f]		Microsomal + soluble fractions Without NADPH[g]	Microsomal + soluble fractions With EDTA[h]	Microsomal + soluble fractions With gallate[i]
Ia	0.19	0.43	1.06	1.06	0.40	0.33	0.12	0.25	0.24
Ib	0.01	0.09	0.16	0.83	0.00	0.05	0.00	0.01	0.00
IIa	0.36	1.19	2.89	1.98	1.12	1.00	1.26	0.26	0.29
IIb	0.03	0.12	0.93	3.97	0.01	0.07	0.03	0.03	0.00
III	0.83	2.58	6.92	8.94	2.35	3.40	0.98	0.33	0.98
Total	1.42	4.41	11.96	16.78	3.88	4.85	2.39	0.88	1.51

Product yield, %[a]

[a] Radioactivity incorporated over control without enzyme. [b] 10,000g pellet. [c] 105,000g pellet. [d] With 0.03 mmol ethyl linoleate. [e] With 0.01 mmol mixed ethyl linoleate hydroperoxides (soybean lipoxygenase derived). [f] 105,000g supernate. [g] NADPH and NADPH-generating system omitted. [h] With 4 mg EDTA. [i] With 0.01 mmol propyl gallate.

quent thermal decomposition of Ib and IIb yielded Ia, IIa, and III as secondary products.[13] In that neither the 5α-hydroperoxide IV nor its unique pyrolysis product cholesta-4,6-dien-3-one[13] was found, the participation of singlet molecular oxygen was excluded from those oxidations.

Subcellular fractions of liver from adult (500 g) male Sprague-Dawley rats incubated with [1,2-³H]cholesterol and a NADPH-generating system (0.015 M Tris–HCl, pH 6.8) at 30° for 2 hr under oxygen yielded the 7-hydroperoxides Ib and IIb, the corresponding 3β,7-diols Ia and IIa, and the 7-ketone III, identified chromatographically.[13b,14] Neither the 5α-hydroperoxide IV nor cholesta-4,6-dien-3-one was detected despite a careful search.

Data of Table I show cholesterol oxidation was greatest in the microsomal and combined microsomal-soluble fractions. Sterol peroxidation was diminished in the absence of NADPH, by boiling the enzyme or by preincubation with trypsin, and was completely inhibited by 1 mM EDTA, by 1 mM propyl gallate, or by dithionite. Carbon monoxide was without effect, as was pretreatment of rats with phenobarbital. Additions of ethyl linoleate or ethyl linoleate hydroperoxides markedly stimulated peroxidation.

The 7-ketone III predominated in most experiments and accounted for approximately half of measured products. The 7α- to 7β-oxygenated product ratio ranged from 1 : 1 to 1 : 5 for the 7-hydroperoxides Ib and IIb and from 1 : 1 to 1 : 10 for the 7-alcohols Ia and IIa, thus emphasizing preference for the quasiequatorial[15] epimers as previously demonstrated in other studies.[6,12,15] The 7-hydroperoxides were not specifically detected in every experiment where the secondary products Ia, IIa, and III were found. However, it is clear that Ia, IIa, and III derived from Ib and IIb and that complex transformations of sterol hydroperoxides occur in liver[11a,c,16] which may or may not be enzymic. The same products Ia, IIa, and III result from pyrolysis[13] and from bimolecular disproportionation[17] of Ib and IIb.

These sterol oxidations by liver microsomes are thus

similar to free radical oxidations of cholesterol by air[12] and by lipoxygenase and peroxidase.[6] The obvious difference is the diminished stability of the initial product hydroperoxides Ib and IIb in the microsomal preparations. Our present data suggest that the lipid peroxidation system involves free radical processes and not singlet molecular oxygen. Neither a putative direct activation of ground-state molecular oxygen nor disproportionation to peroxide anion and singlet molecular oxygen of superoxide radical anion implicated in lipid peroxidations[4c,d,5a,18] is supported by our results.[19] Speculations implicating singlet molecular oxygen in lipid peroxidation in rat liver, indeed in enzyme reactions, should now be viewed with reservation.

Formation of the 7-hydroperoxides Ib and IIb from cholesterol, while formally representing the action of a microsomal dioxygenase, may not be enzymic or involve a sterol–dioxygenase complex. Rather, the cholesterol 7-radical[12,17,20] necessary to formation of Ib and IIb may derive in conjunction with generation of other lipid radicals,[11d] superoxide radical anion,[4c,d,5a,18] hydroxyl radicals resulting from interaction of superoxide and peroxide anions,[21] or yet other radicals.

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Kinetic Analysis of an Intramolecular Addition of a Grignard Reagent to an Alkene

Sir:

Many cyclizations of unsaturated Grignard reagents and (the reverse reactions) ring openings of strained

cyclic Grignard reagents have been studied since the initial observations[1] by Roberts and his coworkers of the interconversion of 3-buten-1-yl and cyclopropylmethyl Grignard reagents.[2-12] Although rates of many of these reactions have been observed, the contributions to these rates by the various organomagnesium species that might be present in the Grignard solutions have yet to be established. In this communication, we report, for the 5-hexen-1-yl (1) Grignard reagent in tetrahydrofuran, a determination of the relative rates of cyclization of RMgBr and RMgR, as well as the value of K_s, the equilibrium constant for the Schlenck equilibrium (eq 1)

$$R_2Mg + MgBr_2 \overset{K_s}{\rightleftharpoons} 2RMgBr \qquad (1)$$
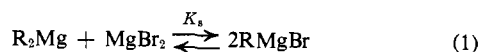
that interconverts these organomagnesium species.

Cyclizations of 1 to 2 were followed by hydrolyzing aliquots of solutions of organomagnesium compounds, followed by glpc analysis of the resulting mixtures of hydrocarbons 3 and 4. Tetrahydrofuran was chosen as
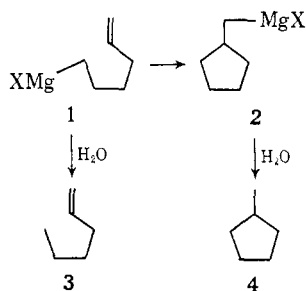
the solvent because organomagnesium compounds generally are monomeric in it.[13] "Single crystal" magnesium was used to prepare Grignard solutions to avoid possible catalysis by impurities in the metal.[14]

Solutions containing only the dialkylmagnesium compound were prepared by dioxane precipitation of $MgBr_2$ from ether solutions of the Grignard reagent,

and the solvent was replaced by tetrahydrofuran. The cyclization at 100° of R (5-hexen-1-yl) to R′ (cyclopentylmethyl) in such solutions followed first-order kinetics.[15] Essentially, identical rate constants (44.8 ± 1.9 × 10⁻⁶ sec⁻¹)[15,16] were obtained at different dialkylmagnesium concentrations in the range 0.08–0.17 M. Although RMgR is the only reactant present initially, as the reaction progresses, RMgR′ is expected to appear, and the rate should depend on both the first and last terms of eq 2. Lack of systematic deviation from

$$\text{rate} = 2k_1[RMgR] + k_2[RMgBr] + k_3[R'MgR] \qquad (2)$$

first-order plots indicates either that (a) $k_1 \simeq k_3$ or (b) that K_s for the equilibration (presumably rapid compared to the isomerization of R to R′)[17] shown in eq 3 is sufficiently large that [R′MgR] remains low. Since

$$2R'MgR \overset{K_s}{\rightleftharpoons} RMgR + R'MgR' \qquad (3)$$
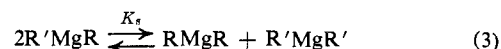
both R and R′ are six-carbon primary groups, it seems far more likely that RMgR and R′MgR are similar in reactivity than different in stability.

The isomerization of R to R′ in solutions of Grignard reagents prepared from 5-hexen-1-yl bromide also followed first-order kinetics. Essentially identical rate constants (11.6 ± 0.3 × 10⁻⁶ sec⁻¹)[15,18] were obtained at Grignard reagent concentrations (expressed as concentrations of R + R′ groups = [R]₀) in the range 0.11–0.40 M. Since this rate is only one-fourth of the rate observed for RMgR, it is likely that at least three-fourths of the R groups are present in RMgBr and that this species is considerably less reactive than RMgR. Consistent with this conclusion, the rate was decreased further by addition of $MgBr_2$ (B = concentration of added $MgBr_2$), although the kinetics continued to show no systematic deviations from first-order behavior.

The kinetic expression for the disappearance of R (or appearance of R′) should be eq 2, but initially, before [R′] is significant, eq 4 should apply. The observation

$$\text{rate} = 2k_1[RMgR] + k_2([R]_0 - 2[RMgR]) \qquad (4)$$

where

$$[RMgR] = \frac{([R]_0 - 2[RMgR])^2}{K_s([RMgR] + B)} =$$

$$\frac{-K_sB - 4[R]_0 \pm \sqrt{K_s(K_sB^2 + 8B[R]_0 + 4[R]_0^2)}}{2K_s - 8}$$

that, within experimental error, first-order kinetics and a constant rate are exhibited throughout each reaction (even those in which B is large and RMgBr may contribute a significant portion of the rate) indicates that eq 4 can be applied not only initially but throughout a reaction. Adherence of the kinetics to eq 4 throughout a reaction requires (if $k_1 \simeq k_3$) that the ratio of R in dialkylmagnesium species (RMgR and R′MgR) to R in alkylmagnesium bromide (RMgBr) remains essentially constant. This would be the case if the values of K_s,

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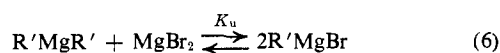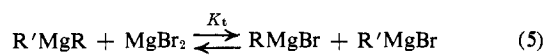
(15) The rate constant derived for each kinetic run was the average of the essentially identical constants obtained for the disappearance of 3 and the appearance of 4.

(16) Average and mean deviation of four kinetic runs.

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(18) Average and mean deviation of six kinetic runs.

K_t, and K_u are similar,[19] reasonable since R and R' are both primary.

$$R'MgR + MgBr_2 \overset{K_t}{\rightleftharpoons} RMgBr + R'MgBr \qquad (5)$$

$$R'MgR' + MgBr_2 \overset{K_u}{\rightleftharpoons} 2R'MgBr \qquad (6)$$

Since k_2, B, and $[R]_0$ are known, k_1 and K_s can be determined from eq 4. This task is simplified by noting that when $[R_2Mg] \ll [R]_0$ and B, then the observed rate constant is approximated by eq 7. Values of $K_s =$

$$k_{obsd} = \frac{2k_1[R]_0}{K_s B} + k_2 \qquad (7)$$

42.1 and of $k_2 = 1.00 \times 10^{-6}$ sec^{-1} are obtained from a plot of $2k_1[R]_0/B$ against k_{obsd}. Using these values in the more exact equation (eq 4) gives calculated rates in good agreement with the observed ones, particularly considering the errors in the approximation used to derive K_s and k_2 and in the experimental determinations of rate, $[R]_0$, and B. The approximate equation (eq 7) should provide too large a value for K_s, and indeed a somewhat better agreement is obtained for slightly smaller values ($K_s \simeq 35$). Since k_2 is much smaller than k_1 and at the largest B (0.18 M) that could conveniently be used, the dialkylmagnesium species still contributed significantly to the rate, the determination of k_2 is subject to considerable error. Nevertheless, a much larger value of k_2 seems unlikely since at the highest value of B that was used, the observed rate was $<2 \times 10^{-6}$ sec^{-1}. A value much nearer zero also seems unlikely, since then the agreement between calculated and observed rates (for any value of K_s) is not as satisfactory at both high B and at $B = 0$. Therefore, we conclude that $k_1 = 44.8 \times 10^{-6}$ sec^{-1}, $k_2 \simeq 1 \times 10^{-6}$ sec^{-1}, and $K_s \simeq 30\text{--}40$.[20]

Previous observations[6] of dependence of rate on the presence of substitutents at $>$C$=$C$<$ and at $-$CMgX suggest that both the alkene and Grignard functions are involved in the rate-determining step. The present results indicate that this involvement can be no more than "first order" in each function. This conclusion is similar to the recent findings by Ashby and his coworkers that the transition states for addition in ether of methylmagnesium chloride or dimethylmagnesium to 2-methylbenzophenone or to benzonitrile contain only one molecule of organomagnesium species and one of ketone or nitrile.[21]

(19) This would be precisely the case if $K_s = K_t/2 = K_u$.

(20) Extrapolation from activation parameters obtained at lower temperatures [M. B. Smith and W. E. Becker, *Tetrahedron*, **23**, 4215 (1967)] gives a value of $K_s = 38$ for the ethyl Grignard reagent (prepared from the bromide) in tetrahydrofuran at 100°.

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[6]Paracyclophane[1]

Benzene is flat. Every undergraduate chemistry student learns early in his career that the extraordinary stability of benzene is associated with the cyclic overlap of six p orbitals. Planarity assures ideal overlap and thus maximizes the attendant stability. Naturally chemists have addressed themselves to the question of how much bending a benzene ring can withstand without giving up its "aromatic character."[2] The most successful experimental approach has been through the construction of [m,n]- and [m]paracyclophanes in which the para positions are bridged with ever-shortening chains.[3] The current limits in the [m] series are marked by Allinger and Walter's synthesis in 1972 of [7]paracyclophane-3-carboxylic acid[4] and our report last year of the formation of the parent hydrocarbon.[5] Here we describe the extension of our method and the construction of [6]paracyclophane.

Ketone 1 was synthesized from cycloheptane carboxaldehyde and methyl vinyl ketone and converted to the tosylhydrazone (2) in unexceptional fashion. Flash pyrolysis of the lithium salt 3 at 250–400° led to 4 as well as alkyl- and alkenylbenzenes. The yield of 4
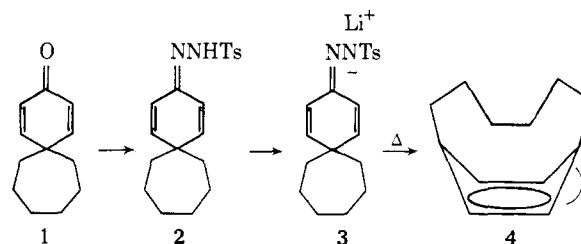
measured by gas chromatography was as high as 5–10%, but the isolated yields were much lower because of the tedious gas chromatography required to separate 4 from other compounds. Purification was not a problem in the [7] series and, with luck, will not generally be a difficulty.[6]

Mass spectrometry established the composition as $C_{12}H_{16}$ (precise mass 160.128135; calcd 160.125194) and revealed the major fragmentation to involve formation of C_8H_8, probably p-xylylene. The ultraviolet spectrum [EtOH, nm (log ϵ): 212 (4.3), 253 (4.0), 296 (2.8)] is only moderately shifted from that of [7]paracyclophane.[5] The 60-MHz, room temperature pmr spectrum showed four approximately four-proton signals at τ 2.83 (singlet), 7.51 (broad triplet), 8.85 (center of multiplet), and 9.67 (center of multiplet). The 100-MHz spectrum is sharper at 90° but broadened considerably and otherwise changed at $-80°$. At the lower temperature the

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