Table I. Summary of Oxygen-18 Kinetic Isotope Effects for Alkoxyl-Labeled *N*-Acyl-L-Tryptophan Ethyl Esters and Methyl Formate

Compound	Reaction	Kinetic isotope effect
II	Chymotrypsin catalyzed hydrolysis	1.0117 ± 0.0004[a]
I	Chymotrypsin catalyzed hydrolysis	1.0180 ± 0.0007[a]
I and II	Hydrazinolysis	1.066 ± 0.002[b,c]
Methyl formate	Hydrazinolysis at pH 7.85	1.0621 ± 0.0008[b,d]
Methyl formate	General base catalyzed hydrolysis	1.0115 ± 0.0002[d]
Methyl formate	Alkaline hydrolysis	1.0091 ± 0.0004[d]

[a] This work determined at pH 6.8. [b] For rate-determining breakdown of the tetrahedral intermediate. [c] C. B. Sawyer and J. F. Kirsch, unpublished results. [d] Reference 7.

was saponified in 3 μl of 6 *M* KOH in methanol for 5 min and the volatile products examined directly by mass spectroscopy. The ratio of ethanol-^{18}O to ethanol-^{16}O was determined as previously described.[5] The percent of total ester remaining at the time of taking of aliquots was determined from the least-squares fit of the integrated form of the Michaelis–Menten rate equation to the progress curve of [S] vs. time.

The kinetic isotope effect for chymotryptic hydrolysis of labeled substrate was determined for I at substrate concentrations of 1.0 m*M* ($12K_m$) and at 0.1 m*M* ($1.2K_m$), and for II at 0.5 m*M* ($2.5K_m$). The oxygen-18 content of unreacted substrate during hydrolysis is plotted as previously described[7] in Figure 1 according to eq. 2 where E, E_0, E^*, and E_0^* are the concentrations of unlabeled and labeled ester at time t and 0. The results are summarized in Table I. The kinetic isotope effect for chymotryptic hydrolysis of I is, within experimental error, independent of initial concentration, as shown in Figure 1.

$$\log (100E^*/E_0^*) - \log (100E/E_0) = (1 - \Delta) \log (100E^*/E_0^*) \quad (2)$$

The 1.2 and 1.8% ^{18}O kinetic isotope effects observed for the chymotrypsin catalyzed hydrolyses of esters I and II are remarkably close to that observed for the general base catalyzed hydrolysis of methyl formate[7] (Table I). This observation supports the proposed general base catalysis mechanism for the acylation of chymotrypsin.[8] We may also calculate the degree of cleavage of the ester bond in the transition state. Studies with methyl formate,[7] I and II,[9] have shown that the ^{18}O kinetic isotope effects for these esters range from 0 to 6.6%, the latter value corresponding to a very late transition state involving complete rate-determining scission of the ester bond. Thus in the chymotrypsin catalyzed reactions the bond order between the acyl carbon and the departing oxygen atoms is reduced about 1.2/6.6 = 18% and 1.8/6.6 = 27% in the transition state for II and I, respectively. This calculation is based on the assumption of a single rate determining transition state. Small kinetic isotope effects can also arise from a reaction pathway involving the formation of one (or more) intermediates whose rates of formation and decomposition exhibit differing sensitivities to the isotopic substitution in question and where both steps are partially rate determining.[10] A likely but by no means established intermediate for the acylation of chymotrypsin by specific *ester* substrates is the tetrahedral adduct formed by attack of Ser-195 on the acyl carbon atom. The available data do not permit differentiation among these two possibilities for this system; however, additional kinetic data employing linear free energy relationships or a second isotopic substitution may resolve this ambiguity.[11] The identical chemical reactivity of I and II (see below) does in our opinion constitute some evidence against different partitioning ratios of a tetrahedral adduct being the explanation for the different values of the observed kinetic isotope effects.

The small difference in observed kinetic isotope effects for I and II may indicate either a later transition state with the same mechanism for I as for II or, possibly, different mechanisms of acylation with the two esters. The latter possibility is intriguing; whereas the ester linkages in I and II are virtually identical (the rates of reaction with OH^- and N_2H_4 are equal within an experimental error of 5%),[9] I but not II can form an oxazoline as an additional intermediate en route to the acyl enzyme. Such an oxazoline has actually been isolated from the reaction of furylacryloyl-tryptophan methyl ester with chymotrypsin at low pH.[12]

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References and Notes

(1) Supported by National Science Foundation Grant No. GB 35573X.
(2) (a) For a recent compilation see J. F. Kirsch, *Annu. Rev. Biochem.*, **42**, 205 (1973); (b) M. H. O'Leary and M. D. Kluetz, *J. Am. Chem. Soc.*, **94**, 3585 (1972).
(3) G. P. Hess, "The Enzymes", Vol. III, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, pp 218–219; F. J. Kezdy, G. E. Clement, and M. L. Bender, *J. Am. Chem. Soc.*, **86**, 3690 (1964).
(4) This assumption, that ^{18}O isotope effects on intermolecular nonbonded interactions are negligible, is supported for example by the fact that the isotope effect on the vapor pressure of $^{12}C^{18}O_2$ relative to $^{12}C^{16}O_2$ is less than 0.01% at 25°. Z. Bilkadi, M. W. Lee, and J. Bigeleisen, *J. Chem. Phys.*, in press.
(5) C. B. Sawyer, *J. Org. Chem.*, **37**, 4225 (1972).
(6) Prepared by the method of E. Fischer, *Chem. Ber.*, **38**, 605 (1905).
(7) C. B. Sawyer and J. F. Kirsch, *J. Am. Chem. Soc.*, **95**, 7375 (1973).
(8) L. W. Cunningham, *Science*, **125**, 1145 (1957); M. L. Bender and F. J. Kezdy, *Annu. Rev. Biochem.*, **34**, 49 (1965); T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. I, W. A. Benjamin, New York, N.Y., 1966, pp 242–258; D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature (London)*, **221**, 337 (1969).
(9) C. B. Sawyer and J. F. Kirsch, unpublished results.
(10) C. R. Hart and A. N. Bourns, *Tetrahedron Lett.*, 2995 (1966); H. H. Huang and F. A. Long, *J. Am. Chem. Soc.*, **91**, 2872 (1969); M. H. O'Leary, *ibid.*, **91**, 6886 (1969); D. G. Graczyk and J. W. Taylor, *ibid.*, **96**, 3255 (1974).
(11) B. W. Palmer and A. Fry, *J. Am. Chem. Soc.*, **92**, 2580 (1970); M. J. Goldstein and G. L. Thayer, Jr., *ibid.*, **87**, 1933 (1965); Z. Bilkadi, R. de Lorimier, and J. F. Kirsch, *ibid.*, in press.
(12) M-A. Coletti-Previero, C. Axelrude-Cavadore, and A. Previero, *FEBS Lett.*, **11**, 213 (1970). See also S. H. Yu and T. Viswanatha, *Eur. J. Biochem.*, **11**, 347 (1969).

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Mechanisms of Grignard Reactions with Ketones. Polar vs. Single Electron Transfer Pathways

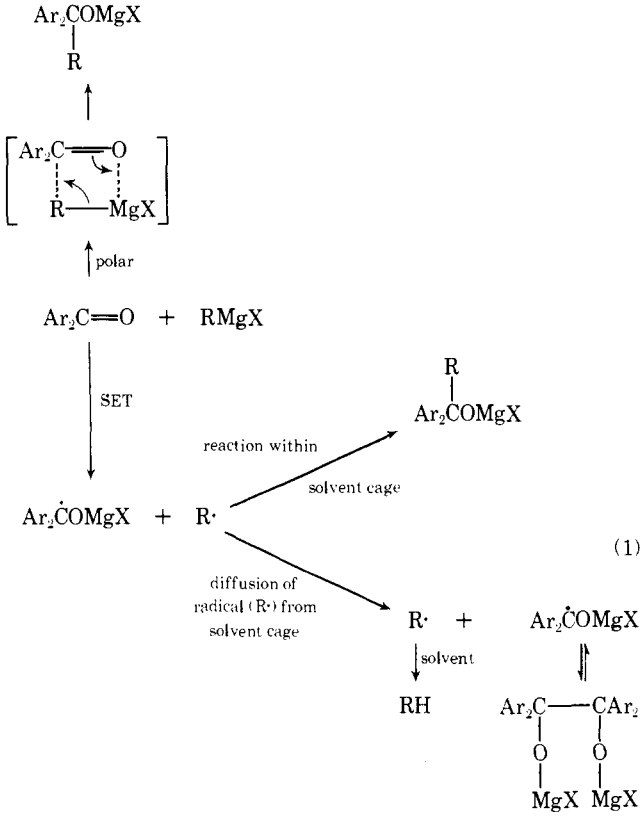
Sir:

In addition to the commonly accepted polar mechanism for the addition of Grignard reagents to ketones, evidence has been accumulating which supports a single electron transfer (SET) process in some cases[1] (reaction 1).[1c] We wish to report preliminary results of the role of ketyls in reactions of Grignard reagents with ketones and the conditions under which the polar or SET mechanisms operate.

Table I. Products from Reaction of CH_3MgBr with 2-Methylbenzophenone in Ether

$[CH_3MgBr]$, M	[K],[a] M	[G]/[K]	Grade of Mg used for CH_3MgBr	% yield 1,2-Addition[b]	% yield Pinacol[c]	% yield 2-Methylbenzhydrol
0.01	1.0	0.01	Doubly sublimed	100	0	0
1.5	1.5	1.0	Doubly sublimed	100	0	0
1.5	0.015	100	Doubly sublimed	89.5	1.5	9
1.5	0.00375	400	Doubly sublimed	62	2	36
1.5	0.00188	800	Doubly sublimed	40	4	56
1.5[d]	0.00375	400	Grignard grade turnings	55	18.5	8
1.5	0.00375	400	Single crystal	99.5	0	0.5

[a] 2-Methylbenzophenone. [b] 1-Phenyl-1-(2-methylphenyl)ethanol. [c] 2,2'-Dimethylbenzopinacol. [d] Products include 18.5% of unknown compound.



The system chosen for study was the reaction of methyl bromide Grignard reagent (designated CH_3MgBr for simplicity) with 2-methylbenzophenone in diethyl ether. Table I shows the variation of products with respect to Grignard: ketone ratio and purity of the magnesium used to prepare the Grignard reagent. Two points are important. First, the amount of by-products (2,2'-dimethylbenzopinacol and 2-methylbenzhydrol) is strongly dependent on the amount of excess Grignard present. Second, different grades of magnesium used to prepare the CH_3MgBr give widely different product distributions. Thus, while 2-methylbenzhydrol is the major by-product using doubly sublimed magnesium, 2,2'-dimethylbenzopinacol becomes predominant with Grignard grade turnings, and practically *no* by-product is formed with single-crystal magnesium even at a Grignard to ketone ratio of 400:1. These results are consistent with previous results[1f,2] from which it was concluded that the by-products were arising via a transition metal catalyzed SET pathway. Hence, as the amount of excess Grignard is increased, the amount of impurities present increases, thus giving rise to larger amounts of by-products.

The effect of added transition metals (in this case iron) is shown in Table II. It is clear from these data that iron in some form does catalyze pinacol formation. No 2-methylbenzhydrol was observed in these reactions.

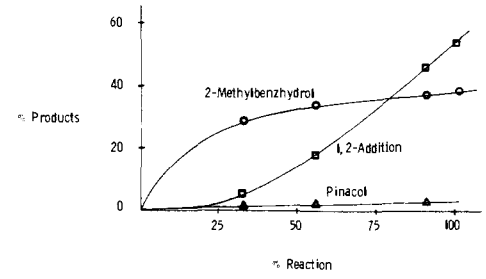


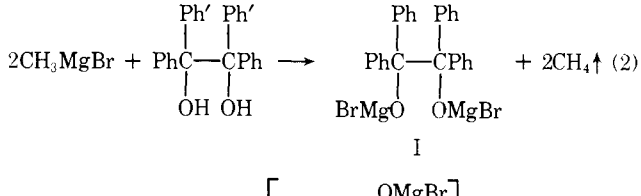
Figure 1. Products vs. time in the reaction of CH_3MgBr (0.50 M) with 2-methylbenzophenone (0.00125 M).

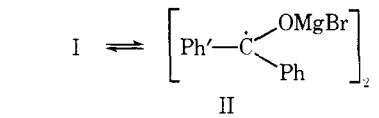
Table II. Effect of $FeCl_3$ on By-Product Formation in the Reaction of CH_3MgBr (0.30 M) with 2-Methylbenzophenone (0.030 M) in Ether

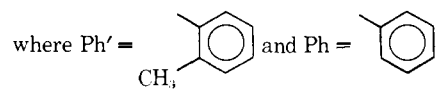
Mole % $FeCl_3$	Fe[a] (ppm)	% yield 1,2-Addition[b]	% yield Pinacol[c]
0	0	100	0
0.005	120	82	18
0.05	1,200	52	48
0.5	12,000	29	71

[a] Relative to Mg in CH_3MgBr. [b] 1-Phenyl-1-(2-methylphenyl)-ethanol. [c] 2,2'-Dimethylbenzopinacol.

Although evidence for ketyl formaton in various Grignard reactions has been presented,[1d,e] the actual role of any ketyl species has not been determined. We prepared ketyl II independently by adding a stoichiometric amount of CH_3MgBr to the pinacol prepared from 2-methylbenzophenone (reactions 2 and 3). The identity and concentration of







II was determined by ESR.[3] The rapidly established equilibrium between I and II had an equilibrium constant (K = [I]/[II(dimer)]) of 9.0 which was constant over the concentration range 6.6×10^{-5} to 1.0×10^{-3} M, indicating the dimeric nature of the ketyl under these conditions. The pink ketyl solution absorbed in the visible region with a λ_{max} at 512 nm and a molar extinction coefficient of 2300. Hydrolysis of the pink solution with saturated aqueous NH_4Cl produced only the starting pinacol.

Table III. Product–Time Study for the Reaction of CH_3MgBr (0.50 *M*) with 2-Methylbenzophenone (0.00125 *M*)[a] in Ether

Reaction time	% unreacted ketone	% yield 1,2-Addition[b]	% yield 2-Methylbenzhydrol	% yield Pinacol[c]	By-product/addition
10 sec	68	2.7	28	1.7	10.8
1 hr	46	18	34	2	2.0
4 hr	11	48	39	2.3	0.85
12 hr	0	56	41	2.5	0.77

[a] At −30°. [b] 1-Phenyl-1-(2-methylphenyl)ethanol. [c] 2,2′-Dimethylbenzopinacol.

Reaction of CH_3MgBr with 2-methylbenzophenone (in stoichiometric amounts or in excess CH_3MgBr) produced almost immediately a pink solution whose visible absorption band (λ_{max} 512 nm) was identical with that of II. However, rather than slowly increasing throughout the reaction, the absorbance at 512 nm quickly reached a maximum and then decreased. The rate of decrease increased with larger excesses of CH_3MgBr. The same behavior was observed when excess CH_3MgBr was added to a solution of II, prepared according to eq 2 and 3. Thus, when the amount of expected by-product was calculated from the absorbance of ketyl at 512 nm, the result agreed with the amount of by-product found experimentally only in the early stages of the reaction, due to the disappearance of ketyl in the presence of excess CH_3MgBr (7% reaction, pinacol calculated from ketyl absorbance, 2.2%; pinacol found on hydrolysis, 2.1%). However, in all cases it was observed that when conditions were such that increased amounts of by-products were observed (increased amounts of excess CH_3MgBr or added $FeCl_3$), a corresponding increase in the amount of ketyl was observed. Thus, while the system is too complicated to establish a precise quantitative relationship between ketyl and by-products, the evidence is clear that the observed by-products do indeed result from the bromomagnesium ketyl intermediate (II).

Evidence for two competing mechanisms (i.e., "polar" vs. SET) was obtained by following the formation of products with time in the reaction of 0.50 *M* CH_3MgBr with 0.00125 *M* 2-methylbenzophenone (Table III, Figure 1). In the early stages of the reaction, 2-methylbenzhydrol is the major product being formed, while in the latter stages (presumably after most of the "catalysts" have been consumed) formation of 1,2-addition product is predominant. Clearly, more than one mechanism is operating. However, when $FeCl_3$ (0.05 mol %) was added to the reaction of 0.20 *M* CH_3MgBr with 0.020 *M* 2-methylbenzophenone, the results were strikingly different. The ratio of by-products to 1,2-addition product was constant throughout the reaction. In this case, there is sufficient "catalyst" present to allow the SET process to compete with the normal polar addition throughout the reaction, thus giving rise to a constant by-product to addition ratio. Although the ratio of the two mechanisms taking place is unknown at present, the $FeCl_3$ catalyzed reaction was found to be definitely faster, and, therefore, the SET mechanism must account for the majority of the products.

Thus, it appears that the addition of CH_3MgBr to 2-methylbenzophenone in ether proceeds via a normal polar mechanism, whereas in the presence of small amounts of transition metal catalysts (e.g., 0.05 mol % of $FeCl_3$) the reaction proceeds via a single electron transfer pathway.[5] The detailed nature of the SET mechanism, including the role of the iron, is presently being studied.

References and Notes

(1) (a) T. Holm and I. Crossland, *Acta Chem. Scand.*, **25**, 59 (1971); (b) J. F. Fauvarque and E. Rouget, *C.R. Acad. Sci., Ser. C*, **267**, 1355 (1968); (c) C. Blomberg, R. M. Salinger, and H. S. Mosher, *J. Org. Chem.*, **34**, 2385 (1969); (d) J. Billet and S. G. Smith, *J. Am. Chem. Soc.*, **90**, 4108 (1968); (e) E. C. Ashby, J. Laemmle, and H. M. Neumann, *J. Am. Chem. Soc.*, **94**, 5421 (1972); (f) E. C. Ashby, H. M. Neumann, F. W. Walker, J. Laemmle, and L. C. Chao, *J. Am. Chem. Soc.*, **95**, 3330 (1973).

(2) E. C. Ashby, F. W. Walker, and H. M. Neumann, *J. Chem. Soc. D*, 330 (1970).

(3) The ESR signal area for the ketyl (obtained by double integration of the derivative spectrum)[4] was compared with that from a standard solution of 2,2,5,5-tetramethylpyrolidine-3-carboxamide-1-oxyl.

(4) S. J. Ward, *J. Sci. Instrum.*, **42**, 769 (1965).

(5) Support of this work by the National Science Foundation is gratefully acknowledged.

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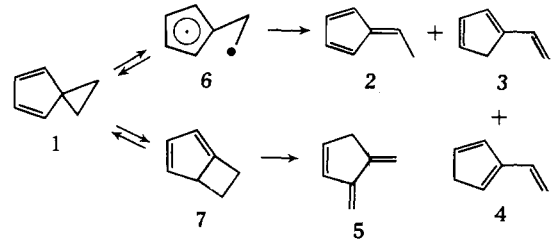
A Skeletally Degenerate Thermal Reorganization of the Spiro[2.4]hepta-4,6-diene Ring System

Sir:

As a part of our continuing interest in the electronic structure and chemical reactivity of the spiro[2.4]hepta-4,6-diene ring system,[1] we now report the observation of a thermal reorganization of this system in which the spiro-[2.4]hepta-4,6-diene ring skeleton remains intact. The thermal reorganization of spiro[2.4]hepta-4,6-diene (**1**) has been reported to yield 6-methylfulvene (**2**), a mixture of the isomeric vinylcyclopentadienes (**3** and **4**), and 3,4-dimethylenecyclopentene (**5**).[2] The overall activation energy of 43.6 kcal/mol is close to that reported for thermal [1,5]-methyl migrations in cyclopentadienes.[3] The results have been interpreted[2] in terms of a rather delicate energy balance between homolytic rupture of the C-1–C-3 bond to provide the cyclopentadienyl–methylene diradical (**6**) and concerted [1,5]-sigmatropic migration of cyclopropyl methylene to afford the intermediate bicyclo[3.2.0]heptadiene (**7**). The pyrolytic interconversion of *cis*- and *trans*-1,2-dimethylspiro[2.4]hepta-4,6-diene at an activation energy of 42.7 kcal/mol[4] is consistent with the intervention of the diradical,[5] and evidence for the facile reverse of the **1** → **7** conversion has recently been reported.[6]



The synthesis of 4- and 5-methylspiro[2.4]hepta-4,6-diene (**8** and **9**) in a 3:2 product ratio (35% overall yield) was readily accomplished by the reaction of methylcyclopentadiene (as a mixture of positional isomers) with 1,2-dibromoethane,[7] and clean separation of the isomeric products was effected by preparative scale GLPC. The assignment of the structures of **8** and **9** is clear from the respective 60-MHz ^1H NMR spectra:[8] **8** ^1H NMR ($CDCl_3$) δ 1.36 (m, 2 H), 1.55 (m, 2 H), 1.70 (d, J = 1.5 Hz, 3 H), 6.18 (m, 1 H), 6.01, 6.46 (AB, 2 H); **9** ^1H NMR ($CDCl_3$) δ 1.51 (s, 4 H), 2.05 (d, J = 1.5 Hz, 3 H), 5.69 (q, J = 1.5 Hz, 1 H), 6.04, 6.35 (AB, 2 H), in which the most significant factor is the chemical shift nonequivalence of the syn and anti cyclopropyl protons induced by the proximity of the C-4 methyl group in **8**.