The Mechanism of Lewis Acid Catalyzed Ene Reactions

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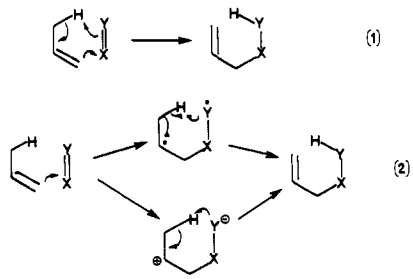
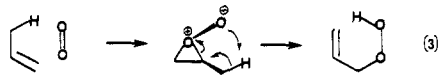
Abstract: Intermolecular kinetic isotope effects with 2,3-dimethyl-2-butene and methylenecyclohexane and intramolecular isotope effects with *gem*-, *trans*-, and *cis*-2,3-dimethyl-2-butene-d_6 establish that the Lewis acid catalyzed ene reactions of methyl propiolate, formaldehyde, and diethyl oxomalonate proceed through a stepwise reaction with rate-determining formation of either (1) a three-membered ring intermediate lacking the geometrical rigidity of perepoxides and related intermediates, (2) a pair of rapidly equilibrating zwitterions, or (3) a π-complex between the ene component and enophile–Lewis acid complex. The intermolecular isotope effects are small and the intramolecular isotope effects with **2** and **3** are large, indicating that the reaction is stepwise. The large intramolecular isotope effects with **1** require that the reaction proceeds through an intermediate that can abstract a hydrogen from either end of the ene component, i.e., one of the three possibilities mentioned above.

The ene reaction traditionally has been thought to proceed through either a concerted pericyclic reaction (eq 1) or a stepwise reaction with a zwitterionic or diradical intermediate (eq 2).[2]



Each of these mechanisms has been established in specific cases. Recent studies have indicated that the ene reaction is mechanistically more diverse. Stephenson has developed a stereochemical isotope test comparing intermolecular kinetic isotope effects with the intramolecular isotope effects obtained with *gem*-, *trans*-, and *cis*-2,3-dimethyl-2-butene-d_6 (**1**, **2**, and **3**, respectively).[3] The use of this isotope test demonstrated that singlet oxygen ene reactions proceed through a third mechanism. The results, in which small isotope effects of $\approx$1.05 are obtained with **3** and in intermolecular competitions and larger isotope effects of $\approx$1.4 are obtained with **1** and **2**, indicate not only that the reactions is stepwise but that it proceeds through a perepoxide-like intermediate which only allows competitive hydrogen abstraction from cis groupings of C–H bonds (eq 3). Seymour and Greene have



used isotope effects obtained with **1**, **2**, and **3** to establish that the ene reactions of triazolinediones and pentafluoronitrosobenzene show similar stereochemical effects and therefore proceed through aziridinium imide and aziridine *N*-oxide intermediates, respectively.[4,5]

Kwart and Brechbiel have explored the temperature dependence of kinetic isotope effects in ene reactions and claimed that the temperature-independent isotope effects in the ene reaction of TsN=S=O (k_H/k_D = 2.86) or diethyl oxomalonate (k_H/k_D = 2.56) with allylbenzene indicate a pseudopericyclic transition state involving nonlinear H transfer.[6] This interpretation has been challenged on theoretical grounds.[7]

Lewis acid catalyzed ene reactions are a versatile synthetic method for the carbofunctionalization of unactivated alkenes.[8] We have developed the Lewis acid catalyzed ene reactions of propiolate esters,[9] α-substituted acrylate esters,[10] acrolein and vinyl ketone derivatives,[11] and unactivated aldehydes[12] into general reactions. Lewis acid catalysis, which causes remarkable rate enhancements, could also effect mechanistic changes.

The high degree of regioselectivity in the Lewis acid catalyzed ene reactions of β-substituted propiolate esters[9b] and α-substituted acrylate esters[10] suggested a concerted mechanism. On the other hand, the concomitant formation of chloro alcohols from Me_2AlCl-catalyzed ene reactions of formaldehyde suggested a stepwise mechanism.[12] The formation of cyclobutenes as side products in the ene reactions of propiolate esters could be accommodated by either competing concerted reactions or branching from a common intermediate. An intramolecular kinetic isotope effect of 2.64 which we observed in the $AlCl_3$-catalyzed ene reaction of **1** and methyl propiolate[9a] also suggested a concerted mechanism (incorrectly!) since a stepwise mechanism appeared to require a larger secondary isotope effect to explain the result than had been observed in the stepwise acylation of alkenes.[13] More recently, Stephenson and Orfanopoulos have found negligible intermolecular and intramolecular primary isotope effects in the $SnCl_4$-catalyzed ene reactions of diethyl oxomalonate and suggested that concerted reaction prevailed with C–H bond breaking only slightly progressed at the transition state.[14] Kwart and Brechbiel have found that the $SnCl_4$-catalyzed reaction of diethyl oxomalonate with allylbenzene gives an oxetane and proposed on this evidence that the rate-determining step in the $SnCl_4$-catalyzed ene reactions of diethyl oxomalonate is the formation of a three-membered complex.[15]

(1) Camille and Henry Dreyfus Teacher-Scholar 1982–1987. Fellow of the Alfred P. Sloan Foundation 1979–1983.
(2) (a) Hoffmann, H. M. R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 556. (b) Oppolzer, W.; Snieckus, V. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 476. (c) Dolbier, W. R., Jr. In "Isotopes in Molecular Rearrangments"; Buncel, E., Lee, C. G., Eds.; Elsevier: Amsterdam, 1975; pp 51–57.
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(4) (a) Cheng, C.-C.; Seymour, C. A.; Petti, M. A.; Greene, F. D.; Blount, J. F. *J. Org. Chem.* **1984**, *49*, 2910. (b) Seymour, C. A.; Greene, F. D. *J. Am. Chem. Soc.* **1980**, *102*, 6385.
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(11) Snider, B. B.; Deutsch, E. A. *J. Org. Chem.* **1983**, *48*, 1822.
(12) (a) Snider, B. B.; Rodini, D. J.; Kirk, T. C.; Cordova, R. *J. Am. Chem. Soc.* **1982**, *104*, 555. (b) Snider, B. B.; Phillips, G. B. *J. Org. Chem.* **1983**, *48*, 464.
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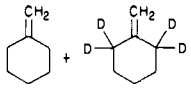
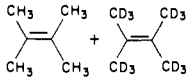
Table I. Kinetic Isotope Effects in Lewis Acid Catalyzed Ene Reactions

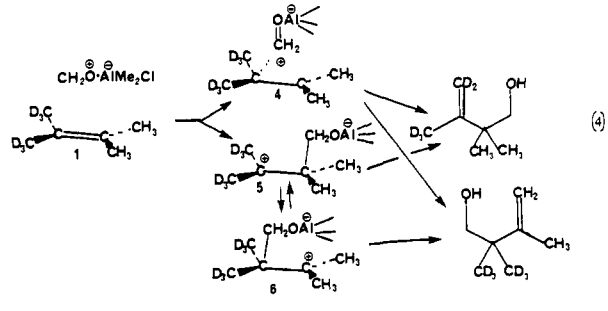
ene	enophile	Lewis acid	yield, %	k_H/k_D
intermolecular				
	CH_2O	Me_2AlCl	64	1.3 ± 0.15
	$HC{\equiv}CCO_2Me$	$EtAlCl_2$	60	1.2 ± 0.15
	$H_2C{=}CClCO_2Me$	$EtAlCl_2$	97	1.1 ± 0.15
	CH_2O	Me_2AlCl		1.4 ± 0.15
	$HC{\equiv}CCO_2Me$	$EtAlCl_2$	59	1.1 ± 0.15
intramolecular				
	CH_2O	Me_2AlCl	57	3.3 ± 0.15
	$HC{\equiv}CCO_2Me$	$EtAlCl_2$	78	2.5 ± 0.15
	$(EtO_2C)_2C{=}O$	$SnCl_4$	90	2.1 ± 0.15
1	$CH_3CO\ SbCl_6$ + $NEt(i\text{-}Pr)_2$			1.9 ± 0.2
	CH_2O	Me_2AlCl	45	2.9 ± 0.15
	$HC{\equiv}CCO_2Me$	$EtAlCl_2$	56	1.8 ± 0.15
2				
	CH_2O	Me_2AlCl	50	2.7 ± 0.15
	$HC{\equiv}CCO_2Me$	$EtAlCl_2$	56	1.6 ± 0.15
	$(EtO_2C)_2C{=}O$	$SnCl_4$	30	1.5 ± 0.15
3				

Results and Discussion

The ambiguities in the above data indicated that a more complete study of intermolecular isotope effects and intramolecular isotope effects with substrates **1**, **2**, and **3** in Lewis acid catalyzed ene reactions (see Table I) could help resolve these discrepancies. Ene reactions in which the competition was intermolecular were run to completion with 1 equiv of enophile and Lewis acid and 5 equiv of each ene component. Ene reactions in which the competition was intramolecular were run to completion with 1 equiv of each component. Ene reactions using formaldehyde–Me_2AlCl,[12a] methyl propiolate–$EtAlCl_2$,[9] methyl α-chloroacrylate–$EtAlCl_2$,[10] diethyl oxomalonate–$SnCl_4$,[16] and acetyl hexachloroantimonate[17] were carried out as previously described. Methyl α-chloroacrylate does not undergo a Lewis acid catalyzed ene reaction with 2,3-dimethyl-2-butene. The rearranged butyrolactone[16] was obtained from the reaction of diethyl oxomalonate with **1** but not with **3**. Fortunately, the isotope effects from the ene reaction with **1** can be determined from analysis of the NMR spectra of the butryolactones. The isomerization of products derived from **1** but not from **3** may be due to different impurities present in the two alkenes as a result of different preparative methods. The isotope effects were determined by analysis of the H and ^2H NMR spectra of the ene adducts.

The results shown in Table I require a stepwise reaction with rate-determining formation of either (1) a three-membered ring or related intermediate, (2) a π-complex, or (3) a pair of rapidly equilibrating zwitterions.[18] Intermolecular kinetic isotope effects of 1.1 to 1.3 in the Lewis acid catalyzed ene reactions of formaldehyde, methyl propiolate, and methyl α-chloroacrylate with methylenecyclohexane and methylenecyclohexane-*2,2,6,6-d*$_4$ are consistent with the expected secondary isotope effects in a stepwise reaction.[2c,13] Similarly small intermolecular kinetic isotope effects were obtained in the Lewis acid catalyzed ene reactions of formaldehyde and methyl propiolate with 2,3-dimethyl-2-butene and 2,3-dimethyl-2-butene-d_{12}. These results clearly indicate that C–H bond breaking is not an important component of the rate-determining step. They are consistent with either a stepwise reaction in which the first step is irreversible or a concerted reaction with a very unsymmetrical transition state. The substantial intramolecular isotope effects obtained in the Lewis acid catalyzed ene reactions of formaldehyde and methyl propiolate with **2** and **3**, coupled with the small intermolecular isotope effects, indicate that the reaction is stepwise with proton transfer following the rate-determining step. In an intramolecular competition such as that in the ene reactions of **2** and **3**, an isotope effect will still be observed if the hydrogen transfer occurs after the rate-determining step. If the reaction were concerted, the intermolecular and intramolecular isotope effects should be of similar magnitude.

The large isotope effects obtained with methyl propiolate or formaldehyde and **1** are most significant. They are not consistent with a simple stepwise mechanism, proceeding through a zwitterionic intermediate, which cannot have a primary isotope effect and should have secondary isotope effects on the order of 1.1–1.4 as observed in the intramolecular competitions. Reversible formation of the intermediate followed by loss of a proton in the rate-determining step is excluded by the much smaller intermolecular isotope effect. These results require the formation of a three-membered-ring intermediate (**4**), which can also be thought of as a π-complex, or the kinetically equivalent pair of rapidly equilibrating zwitterions (**5** and **6**) as indicated for formaldehyde in eq 4. Similar intermediates proposed for the ene reactions with methyl propiolate are not illustrated. The slightly larger isotope effects obtained with **1** than with **2** or **3** probably result from secondary isotope effects.



(4)

A referee has suggested that the isotope effects obtained with **1** are due to protonation of **1** to give a cation which can undergo scrambling of methyl and deuteromethyl groups[19] followed by loss of a proton to give a mixture of **1**, **2**, and **3**. This possibility can be excluded even though the control experiment, examination of recovered alkene, cannot be carried out since **1**, **2**, and **3** are

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(17) Hoffmann, H. M. R.; Tsushima, T. *J. Am. Chem. Soc.* **1977**, *99*, 6008.

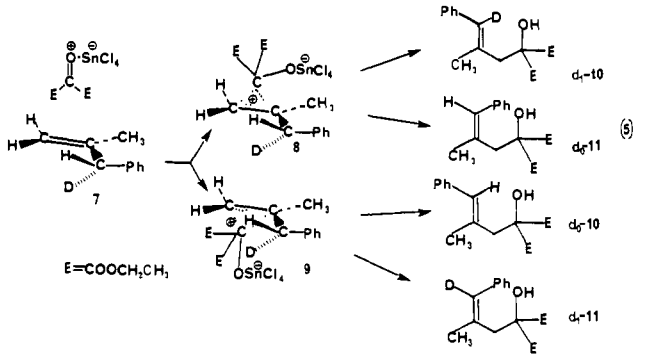
(18) A referee has suggested that a stable ene–enophile–Lewis acid complex is formed and that hydrogen transfer actually takes place during the aqueous quench. This can be excluded since we have monitored these reactions by NMR spectroscopy and established that an ene adduct–Lewis acid complex is formed prior to the aqueous quench.[9a]

(19) Saunders, M.; Vogel, P. *J. Am. Chem. Soc.* **1971**, *93*, 2561.

indistinguishable spectroscopically. We have carried out ene reactions, under identical conditions, using alkenes such as β-pinene and methylenecyclohexane which rearrange during a protonation–deprotonation sequence.[9,12] No rearrangement is observed in these cases since the alkylaluminum halides used as Lewis acid catalysts are also proton scavengers so that protonation of alkenes does not occur during these ene reactions.[20] It is therefore implausible to postulate protonation of **1**. Furthermore, the isotope effect observed with **1** is larger than that obtained with **2** and **3** and the NMR spectra show relative intensities consistent with products which would be obtained from **1** but not with those from **2** and **3**. The possibility of methyl scrambling in an intermediate such as **5** or **6** can be excluded since the initial methyl shift would be followed by a hydride shift from the oxymethyl group to give 2,3,3-trimethylbutanal,[21] which is not observed.

Since a significant isotope effect is still observed with **3**, the oxygen must be able to abstract a proton from any of the four methyl groups if the three-membered-ring intermediate **4** is formed. These Lewis acid catalyzed ene reactions differ markedly from the ene reactions of singlet oxygen, triazolinediones, and pentafluoronitrosobenzene which show a significant isotope effect with **2** but not with **3**. However, these enophiles all have a lone pair on the atom bonding to the ene component which allows the formation of "stable" perepoxide, aziridinium imide, and aziridine *N*-oxide intermediates in these ene reactions. The slightly larger isotope effects obtained with **2** than with **3** indicate that intermediate **4**, or **5** and **6**, shows a slight preference for choosing between cis rather than trans substituents.

We have also examined intramolecular isotope effects in the $SnCl_4$-catalyzed ene reactions of diethyl oxomalonate with **1** and **3**. The isotope effects of 2.1 obtained with **1** and 1.5 obtained with **3** are in line with the results obtained with formaldehyde and methyl propiolate and indicate that the reaction is stepwise. These results appear to contrast with an isotope effect of 1.1 obtained by Stephenson and Orfanopoulos in the ene reaction of diethyl oxomalonate with **7** and used as evidence for a concerted mechanism with C–H bond breaking only slightly progressed in the transition state.[14] However, closer examination as shown in eq 5 indicates that Stephenson's result is consistent with the rate-determining formation of a three-membered-ring intermediate.

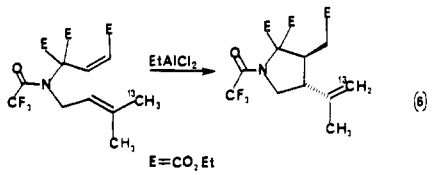


(5)

10-d_0 and **10**-d_1 will be formed from diastereometric intermediates. Intermediate **8** can only react to give **10**-d_1 or **11**-d_0, while the diastereomeric intermediate **9** can only react to give **10**-d_0 or **11**-d_1. Since **11** is known to be formed in only trace amounts, intermediates **8** and **9** which should be formed in almost equal amounts in the rate-determining step must react further to give almost equal amounts of **10**-d_1 and **10**-d_0. These results establish that **7** is not a useful substrate for distinguishing between a concerted mechanism and a stepwise mechanism with rate-determining formation of a three-membered-ring intermediate, although it does rule out a simple stepwise mechanism with a freely rotating intermediate.

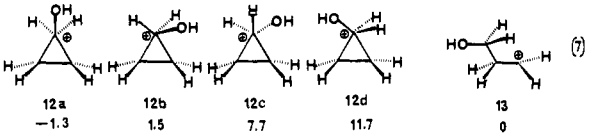
The Friedel–Crafts acylation of **1** by acetyl hexachloroantimonate[17] gives a kinetic isotope effect of 1.9. This result allows us to extend Beak and Berger's conclusion that the acylation is stepwise[13] to include the rate-determining formation of a three-membered ring or rapidly equilibrating zwitterion. 1,2-Acyl migration in similar zwitterions is well precedented.[22]

A stepwise mechanism with rate-determining formation of a three-membered-ring intermediate or kinetically equivalent rapidly equilibrating pair of zwitterions can accommodate the high degree of stereo- and regioselectivity which we[8-12] and others[23,24] have observed in Lewis acid catalyzed ene reactions and used incorrectly as evidence for concerted reactions. Such selectivity is inconsistent with a freely rotating zwitterion but perfectly compatible with a geometrically well-defined intermediate. For instance, the complete selectivity for transfer of a hydrogen from the *trans*-methyl group observed by Oppolzer in the Lewis acid catalyzed ene reaction shown in eq 6[23] rules out a freely rotating zwitterion, but not the mechanism we have proposed.



(6)

The proposed stepwise mechanism has a firm theoretical basis. Schleyer has calculated the energies of cations similar to **4** and **5** formed from the interaction of ethylene and protonated formaldehyde.[25] These calculations suggest that the bridged ion **12** which corresponds to **4** is slightly more stable than the open ion **13** corresponding to **5** and **6**. (See eq 7, relative STO-3G energies in kcal/mol are given.) Furthermore, several configurations of the bridged ion **12** are calculated to have similar energies. If the bridged ion **4** can equilibrate among similar configurations it then should be able to abstract a proton from any of the four methyl groups as is observed. Dewar and Reynolds have proposed that π-complexes similar to **4** are intermediates in cation–olefin cyclizations.[26]



(7)

Conclusion

The results presented here firmly establish that these Lewis acid catalyzed ene reactions do not proceed through either a concerted pericyclic mechanism or a simple stepwise mechanism but rather through a more complex stepwise mechanism. The rate-determining step is the formation of either (1) a three-membered-ring intermediate lacking the geometrical rigidity of perepoxides and related intermediates, (2) a pair of rapidly equilibrating zwitterions, or (3) a π-complex between the ene component and enophile–Lewis acid complex. Although this conclusion can be applied with certainty only to 2,3-dimethyl-2-butene since changing the substitution pattern of the alkene may change the mechanism, it is consistent with all available data on the mechanism of the Lewis acid catalyzed ene reaction.

Finally, experimental schemes designed to distinguish only between concerted and simple stepwise mechanisms with a freely rotating intermediate in Lewis acid catalyzed ene reactions must now be extended to consider the more complex stepwise mechanism in which the rate-determining step is one of the three kinetically

(20) Snider, B. B.; Rodini, D. J.; Kirk, T. C.; Deutsch, E. A.; Cordova, R.; Price, R. T. *Tetrahedron* **1981**, *37*, 3927.
(21) Snider, B. B.; Karras, M.; Price, R. T.; Rodini, D. J. *J. Org. Chem.* **1982**, *47*, 4538.
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equivalent possibilities mentioned above. The observation of an intramolecular kinetic isotope effect with **1** (or equivalent substrates) and the absence of an intermolecular isotope effect indicate that this third mechanism is operative.

Experimental Section

General Procedures. NMR spectra were recorded on Varian EM-390, Perkin-Elmer R32, and Varian XL-300 NMR spectrometers in $CDCl_3$. Chemical shifts are reported in δ (ppm downfield from Me_4Si). A 40–60 s delay was used between pulses when recording FT H and ^2H NMR spectra to ensure complete relaxation of the nuclei and accurate integration. All of the following isotope effect determinations were carried out at least twice. Representative procedures are given. MPLC refers to medium pressure liquid chromatography on silica gel.

Preparation of Deuterated Alkenes. 2,3-Dimethyl-2-butene-d_{12},[27] methylenecyclohexane-2,2,6,6-d_4,[13] and *gem*-2,3-dimethyl-2-butene-d_6 (**1**)[28] were prepared by literature procedures. *cis*- and *trans*-2,3-dimethyl-2-butene-d_6 (**2** and **3**) were prepared by Greene and Seymour's modification[4a,29] of the procedure of Grdina, Orfanopoulos, and Stephenson.[3a,30]

Determination of the Intermolecular Isotope Effect in the Ene Reaction of Methylenecyclohexane and Formaldehyde. Reaction of a 1.16:1 mixture of methylenecyclohexane-d_0 and -d_4 (480 mg, 4.9 mmol), paraformaldehyde (14 mg, 0.45 mmol), and Me_2AlCl (0.25 mL of 1.9 M in hexane, 0.5 mmol) for 3 h at 25 °C as previously described[12a] followed by MPLC (10:1 hexane-EtOAc) gave 38 mg (64%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 5.47 (br, 1, d_0) and 3.62 (t, 2, J = 7.5 Hz, d_0 and d_3).

Determination of the Intermolecular Isotope Effect in the Ene Reaction of Methylenecyclohexane and Methyl Propiolate. Reaction of a 1.16:1 mixture of methylenecyclohexane-d_0 and -d_4 (430 mg, 4.4 mmol), methyl propiolate (28 mg, 0.34 mmol), and $EtAlCl_2$ (0.35 mL of 1.44 M in hexane, 0.5 mmol) for 31 h at 25 °C as previously described[9] followed by MPLC (5:1 hexane–ether) gave 38 mg (60%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 6.94 (dt, 1, J = 16, 6.6 Hz, d_0, and br, 1, d_4), 5.80 (d, 1, J = 16 Hz, d_0), 5.47 (br, 1, d_0), and 3.76 (s, 3, d_0 and d_4).

Determination of the Intermolecular Isotope Effect in the Ene Reaction of Methylenecyclohexane and Methyl Chloroacrylate. Reaction of a 1.16:1 mixture of methylenecyclohexane-d_0 and -d_4 (295 mg, 3 mmol), methyl chloroacrylate (36 mg, 0.3 mmol), and $EtAlCl_2$ (0.21 mL of 1.44 M in hexane, 0.31 mmol) in 5 mL of benzene for 27 h as previously described[10] followed by MPLC (7:1 hexane–EtOAc) gave 90 mg (97%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 5.40 (br, 1, d_0), 4.22 (br, 1, d_0), and 3.76 (s, 3, d_0 and d_4).

Determination of the Intermolecular Isotope Effect in the Ene Reaction of 2,3-Dimethyl-2-butene and Formaldehyde. Reaction of a 1.07:1 mixture of 2,3-dimethyl-2-butene-d_0 and -d_{12} (71 mg, 0.78 mmol), paraformaldehyde (2.4 mg, 0.08 mmol), and Me_2AlCl (0.06 mL of 1.6 M in hexane, 0.1 mmol) as previously described[12a] followed by flash chromatography on silica gel (3:1 pentane–EtOAc) gave a mixture of pure ene adduct and residual solvent. Due to the volatility of the product the residual solvent was removed by dissolution in CCl_4 and partial evaporation of the solvent under reduced pressure to give 23.4 mg of ene adduct containing some CCl_4. The isotope effect was determined from integration of the NMR signals at 4.94 (br s, 1, d_0), 4.84 (br s, 1, d_0), and 3.40 (s, 2, d_0 and d_{11}).

Determination of the Intermolecular Isotope Effect in the Ene Reaction of 2,3-Dimethyl-2-butene and Methyl Propiolate. Reaction of a 1.07:1 mixture of 2,3-dimethyl-2-butene-d_0 and -d_{12} (71 mg, 0.78 mmol), methyl propiolate (6.6 mg, 0.08 mmol), and $EtAlCl_2$ (0.07 mL of 1.44 M in hexane, 0.1 mmol) as previously described[9] followed by flash chromatography on silica gel (10:1 pentane–ether) gave 8.2 mg (59%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 6.97 (d, 1, J = 16 Hz, d_0, and t, 1, $J_{H,D}$ = 2.5 Hz, d_{12}), 5.78 (d, 1, J = 16 Hz, d_0), 4.83 (br, 1, d_0), 4.81 (br, 1, d_0), and 3.74 (s, 3, d_0 and d_{12}).

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 1 and Paraformaldehyde. Reaction of **1** (40 mg, 0.45 mmol), paraformaldehyde (17 mg, 0.6 mmol), and Me_2AlCl (0.76 mL of 1.93 M in hexane, 1.5 mmol) in 15 mL of CH_2Cl_2 as previously described[12a] followed by MPLC (2:1 hexane–EtOAc) gave 32 mg (57%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 4.86 (br, 2, H transfer), 3.37 (d, 2, J = 4 Hz, H and D transfer), 1.80 (s, 3, H transfer), and 1.10 (s, 6, D transfer).

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 1 and Methyl Propiolate. Reaction of **1** (144 mg, 1.6 mmol), methyl propiolate (125 mg, 1.5 mmol), and $EtAlCl_2$ (0.72 mL of 1.44 M in hexane, 1.0 mmol) in 1 mL of benzene for 5 d at 25 °C as previously described[9] followed by flash chromatography on silica gel (9:1 hexane–EtOAc) gave 218 mg (78%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 6.93 (d, 1, J = 16 Hz, H transfer and br, 1, D transfer), 5.73 (d, 1, J = 16 Hz, H transfer), 4.80 (s, 2, H transfer), 3.72 (s, 3, H and D transfer), 1.67 (s, 3, H transfer), and 1.17 (s, 6, D transfer).

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 1 and Diethyl Oxomalonate. Reaction of **1** (10 mg, 0.12 mmol), freshly distilled diethyl oxomalonate (22 mg, 0.12 mmol), and $SnCl_4$ (33 mg, 0.12 mmol) in 2 mL of anhydrous benzene for 6 min at 0 °C followed by addition of the reaction mixture to 10% hydrochloric acid and normal workup[16] gave 38 mg of crude product. Flash chromatography on silica gel (10:1 hexane–EtOAc) gave 25.3 mg (90%) of the rearranged butyrolactones, ethyl tetrahydro-3-hydroxy-4,4,5,5-tetramethyl-2-oxofuran-3-carboxylate. The isotope effect was determined from integration of the NMR signals at 1.43 (s, 3, H transfer), 1.33 (s, 3, H transfer), 1.03 (s, 3, D transfer), and 1.00 (s, 3, D transfer).

Determination of the Intramolecular Isotope Effect in Acetylation of 1.[17] Alkene **1** (235 mg, 2.6 mmol) and ethyldiisopropylamine (1.008 g, 7.5 mmol) were dissolved in 10 mL of CH_2Cl_2 and cooled to −55 °C. The resulting solution was added via syringe to a suspension of acetyl hexachloroantimonate (2.8 g, 7.4 mmol) in 30 mL of CH_2Cl_2 at −55 °C. The solution was stirred for 1 h at −55 °C and 4 h at −20 °C and worked up to give 265 mg of crude product. Evaporative distillation (25 °C, 10 torr) into a liquid nitrogen cooled receiver gave 0.135 g of partially purified product. Since none of the impurities resulted from reaction with **1**, the isotope effect was determined from the integration of the ^2H NMR signals of this mixture at 4.87 (br, 2 D, D transfer), 1.72 (s, 3 D, D transfer), and 1.14 (s, 6 D, H transfer).

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 2 and Paraformaldehyde. Reaction of **2** (24 mg, 0.20 mmol), paraformaldehyde (7 mg, 0.23 mmol), and Me_2AlCl (0.30 mL of 1.93 M in hexane, 0.6 mmol) in 2 mL of CH_2Cl_2 as previously described[12a] followed by flash chromatography on silica gel (3:1 hexane–EtOAc) gave 14 mg (45%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 4.83 (br, 2, H transfer), 3.42 (s, 2, H and D transfer), 1.75 (s, 3, D transfer), and 1.05 (s, 3, H and D transfer). An identical result was obtained by integration of the ^2H NMR signals at 4.86 (br, 2 D, D transfer), 1.75 (s, 3 D, H transfer), and 1.05 (s, 3 D, H and D transfer).

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 2 and Methyl Propiolate. Reaction of **2** (30 mg, 0.34 mmol), methyl propiolate (42.6 mg, 0.5 mmol), and $EtAlCl_2$ (0.21 mL of 1.44 M in hexane, 0.3 mmol) in 1 mL of benzene for 5 days at 25 °C as previously described,[9] followed by flash chromatography on silica gel (9:1 hexane–EtOAc) gave 33 mg (56%) of pure ene adduct. The isotope effect was determined from integration of the NMR signals at 6.95 (d, 1, J = 16 Hz, H transfer and br, 1, D transfer), 5.78 (d, 1, J = 16 Hz, H transfer), 4.80 (s, 2, H transfer), 3.72 (s, 3, H and D transfer), 1.68 (s, 3, D transfer), and 1.18 (s, 3, H and D transfer). An identical result was obtained by integration of the ^2H NMR signals at 5.78 (br, 1 D, D transfer), 4.80 (s, 2 D, D transfer), 1.68 (s, 3 D, H transfer), and 1.18 (s, 3 D, H and D transfer).

Determination of the Intermolecular Isotope Effect in the Ene Reaction of 3 and Paraformaldehyde. Reaction of **3** (53 mg, 0.60 mmol), paraformaldehyde (21 mg, 0.70 mmol), and Me_2AlCl (0.93 mL of 1.93 M in hexane, 1.1 mmol) in 8 mL of CH_2Cl_2 as previously described[12a] followed by MPLC (3:1 hexane–EtOAc) gave 35 mg (50%) of pure ene adduct. The isotope effect was determined as described above for the ene reaction of **2**.

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 3 and Methyl Propiolate. Reaction of **3** (53 mg, 0.60 mmol), methyl propiolate (75 mg, 0.9 mmol), and $EtAlCl_2$ (0.83 mL of 1.44 M in hexane, 1.2 mmol) in 5 mL of benzene for 7 days at 25 °C as previously described[9] followed by flash chromatography on silica gel (10:1 hexane–EtOAc) gave 58 mg (56%) of pure ene adduct. The isotope effect was determined as described above for the ene reaction of **2**.

Determination of the Intramolecular Isotope Effect in the Ene Reaction of 3 and Diethyl Oxomalonate. Reaction of **3** (80 mg, 0.90 mmol), freshly distilled diethyl oxomalonate (157 mg, 0.90 mmol), and $SnCl_4$ (260 mg, 1.0 mmol) in 10 mL of anhydrous benzene for 6 min at 0 °C followed by addition of the reaction mixture to 10% hydrochloric acid and normal workup[16] gave 286 mg of crude product. Flash chromatog-

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raphy on silica gel (10:1 hexane-EtOAc) gave 72 mg (30%) of pure ene adduct. The isotope effect was determined by integration of the NMR signals at 4.87 (br, 2, H transfer), 4.23 (q, 4, J = 6.6 Hz, H and D transfer), 1.80 (s, 3, D transfer), and 1.37 (s, 3, H and D transfer). An identical result was obtained by integration of the ^{2}H NMR signals at 4.87 (s, 2 D, D transfer), 1.80 (s, 3 D, H transfer), and 1.37 (s, 3 D, H and D transfer).

Acknowledgment. We are grateful to the National Institutes of Health for generous financial support.

Arsenite-Inhibited Xanthine Oxidase—Determination of the Mo-S-As Geometry by EXAFS

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Abstract: The interaction of arsenite with oxidized, partially reduced, and fully reduced forms of xanthine oxidase has been studied by X-ray absorption spectroscopy at the arsenic and molybdenum K edges. Clear evidence for a Mo-As interaction at 3.00 Å is observed in the molybdenum EXAFS of the fully reduced ternary complex consisting of xanthine oxidase, arsenite, and the inhibitor 8-bromoxanthine. An essentially identical Mo-As distance of 3.02 Å was found for the Mo(V) complex. The accuracy of distances reported is expected to be ±0.03 Å for first coordination sphere bonds and ±0.05 Å for longer interactions. Surprisingly, no distinct features attributable to a Mo-As interaction are observed in the EXAFS of the oxidized enzyme, from either the molybdenum or the arsenic point of view. Furthermore, no Mo-Br interaction was observed when 8-bromoxanthine was present. For oxidized xanthine oxidase, terminal Mo=O and Mo=S bonds were observed at distances of 1.63 and 2.15 Å in the arsenite complex and 1.67 and 2.15 Å in the oxidized control sample. An additional set of thiolate-like ligands was found at 2.43 and 2.44 Å in the arsenite-inhibited and control samples, respectively. For the oxidized arsenite complex, the arsenic EXAFS suggested binding by three oxygen ligands with an average As-O distance of 1.78 Å. In the fully-reduced arsenite plus 8-bromoxanthine complex, a short Mo=O bond with a length of 1.69 Å was observed, comparable to the Mo=O distance of 1.67 Å in the uncomplexed reduced enzyme. In both cases, terminal Mo=S interactions were no longer evident. A strong Mo-S interaction, best fit by three sulfurs, was observed at average distances of 2.38 and 2.39 Å in the reduced and reduced-ternary complex data, respectively. For the latter complex, an As-S distance of 2.27 Å was calculated from the arsenic EXAFS, corresponding to at least one and more likely two As-S interactions. A weak As-O interaction at 1.78 Å was also observed. Assuming Mo-S-As bonding, and ±0.05 Å accuracy for the distances revealed by EXAFS, a Mo-S-As angle of 80 ± 4° can be derived.

Introduction

Arsenite is a potent inhibitor of the enzyme xanthine oxidase,[2] and recent progress in interpretation of the EPR spectra of the Mo(V)-arsenite complex has stimulated interest in the nature of the Mo-As interaction.[3-5] The EPR spectra of the arsenite complex are affected by the presence of substrates and inhibitors.[5] It has also been shown that 8-bromoxanthine, possessing a heavy atom at the position otherwise hydroxylated by enzyme, binds to the molybdenum center of xanthine oxidase[6] and perturbs the Mo EPR signal of arsenite-complexed enzyme (R. Hille, unpublished). However, although the EPR spectra are rich in information about the nature of the ligands present in the Mo(V) complex, they do not yield bond lengths or angles, nor can they address the Mo(IV) and Mo(VI) oxidation states. The ternary complex consisting of arsenite-inhibited xanthine oxidase plus 8-bromoxanthine contains Mo, As, and Br centers in reasonable proximity and therefore appeared to be an ideal candidate for investigation by X-ray absorption spectroscopy.[7] This technique has already been used to probe the molybdenum site in uninhibited milk xanthine oxidase and liver xanthine dehydrogenase,[8-10] as well as other enzymes containing molybdenum[11] or other metals.[12] In this paper, structural results are presented concerning the environment of xanthine oxidase molybdenum in the presence of arsenite under oxidized, partially reduced, and fully reduced conditions. The arsenic environment under oxidized or fully reduced conditions has also been probed.

Experimental Section

Sample Preparation and Handling. Xanthine oxidase was prepared by a modification of a previously described procedure and assayed as described therein.[13] Enzyme thus obtained was generally in the range of 60-70% active and contained corresponding amounts of the inactive desulfo form of the enzyme. The active enzyme was separated from the inactive with use of the folate affinity column procedure of Nishino et al.[14] and for the present studies was enriched to greater than 92% active enzyme. Active enzyme thus prepared was concentrated to approximately 150 mg/mL with use of an Amicon PM-10 membrane. In all cases the buffer used was 0.1 M aqueous Bicine, pH 8.5. Exact concentration was determined by the absorbance at 450 nm of a 1:100 dilution of this material, which was again assayed to determine that activity had not been lost. Appropriate amounts of arsenite and/or 8-bromoxanthine were then added to known volumes of the enzyme solution and allowed to incubate for 0.5 to 1 h. Samples to be examined in the oxidized state were then transferred to lucite cells, frozen slowly with dry ice, and stored in liquid nitrogen. Those samples to be reduced were placed in the sample cells after Amicon concentration under nitrogen, treated with a twofold excess of solid sodium dithionite, capped with serium stoppers, and incubated for 1 h. At the end of the incubation

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