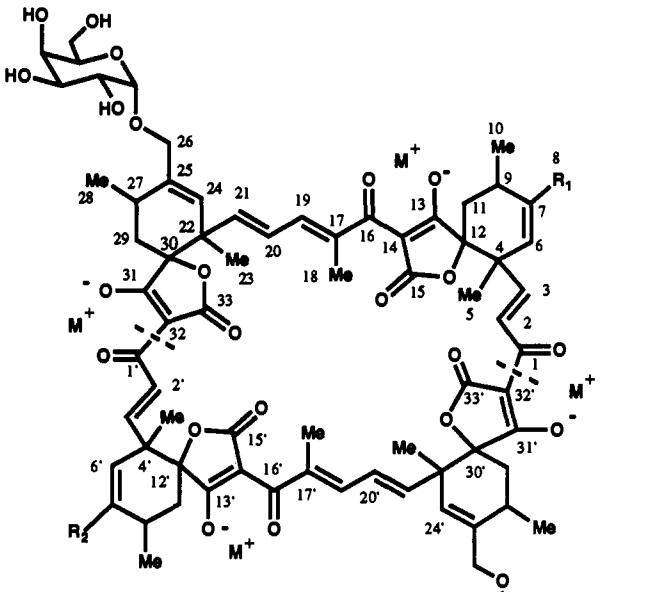


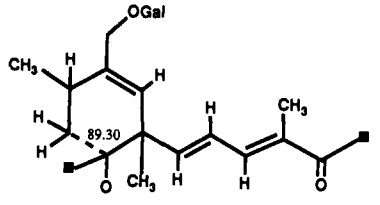
1; $R_1 = R_2 = CHO$

2; $R_1 = CH_2OH$, $R_2 = CHO$

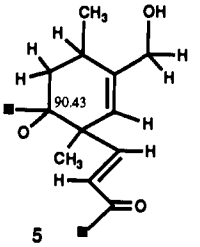
3; $R_1 = R_2 = CH_2OH$

M = Na^+, K^+, Ca^{++}

to the remaining six carbons, were quite puzzling: (1) The IR spectrum of **3** exhibits intense and broad bands at 1620 and 1450 cm^{-1}, which are reminiscent of a carboxylate group. The bands, however, show no change on acidification (1 N HCl). (2) The ^{13}C NMR signals of the six unassigned carbons appeared to be composed of two sets of carbonyl and olefin groups (δ 201.29/201.17; 179.68/178.51; 101.73/99.95), which indicates the existence of two similar functional groups. Most of these carbons, however, exhibit no correlation peaks to any protons in the COLOC[5] or LSPD[6] spectra. (3) The UV maximum at 300 nm (ϵ 50000) [another at 236 nm (ϵ 57000); neither of which changes on addition of 1 N NaOH solution] cannot be interpreted as being due to the chromophores which are present in the partial structures **4** and **5**.



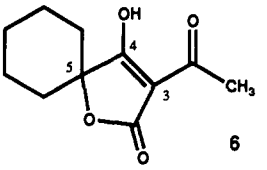
4



5

Chemical reactions a–c were carried out to uncover the structure of the hidden atoms. (a) Sodium borohydride reduction of **3** gave the product [C(1)=O and C(16)=O are reduced], which exhibits a UV maximum at 258 nm. (b) Hydrogenated product (Pd/C, 48 h) [C(2)=C, C(17)=C, and C(20)=C are saturated, but C(6)=C and C(24)=C were left intact, possibly owing to steric hindrance] shows absorption maxima at 233 and 267 nm and strong IR bands at 1620 and 1460 cm^{-1}. (c) On further treatment of the hydrogenation product with sodium borohydride, the UV maximum shifted to 258 nm.

The UV properties of the hydride reduction products are reminiscent of tetronate moieties (λ_{max} ca. 260 nm).[7] Therefore, the original chromophore was a 3-acyltetronate group in which only the acyl group is reduced by the hydride reducing agent, and the tetronate subunit is intact. The UV maxima of the hydrogenation product (b) resemble those of a sodium 3-acetyltetronate (λ_{max} 231, 256 nm).[8] The ^{13}C NMR signals of the aforementioned six carbons suggested the presence of two tetronate moieties. The chemical shifts (δ 90.43/89.30) of the two quaternary carbons can be assigned to sp^3 carbons linked by an oxygen atom [C(12), C(30)]. Therefore, structure **3** was deduced for quartromicin A_3. The IR spectrum of the sodium salt of synthetic **6**[9] exhibits intense bands at 1630 and 1450 cm^{-1}. Its ^{13}C NMR signals[10] are in good agreement with the corresponding signals of **3**.



6

The structures of quartromicins A_1 (**1**) and A_2 (**2**) have been determined by analogous spectroscopic analyses and chemical correlation with **3**.[11]

There are very few macrocyclic antibiotics whose carbon frameworks are composed of only C–C linkages.[12] Quartromicins A_1–A_3 are the largest members of this category.

Quartromicins A_1–A_3 exhibit good in vitro antiviral activity against herpes simplex virus type 1 infected on Vero cells with ID_{50} values around 10 μg/mL by the cytopathic effect reduction assay.

Supplementary Material Available: Listings of ^1H and ^{13}C NMR data for the aglycon and galactose parts of **3** (2 pages). Ordering information is given on any current masthead page.

(5) Kessler, H.; Griesinger, C.; Zarbock, J.; Looslie, H. R. *J. Magn. Reson.* **1984**, *57*, 331.
(6) Seto, H.; Sakai, T.; Yonehara, H.; Uzawa, J. *Tetrahedron Lett.* **1978**, 923.
(7) Scott, A. I. In Interpretation of the Ultraviolet Spectra of Natural Products; International Series of Monographs on Organic Chemistry, Vol 7; Barton, D. H. R., Doering, W., Eds.; Pergamon Press: Oxford, 1964; p 240.
(8) De Keukeleire, D.; De Taeye, L.; Verzele, M. *Tetrahedron* **1976**, *32*, 2932.
(9) Lacey, R. N. *J. Chem. Soc.* **1954**, 832.
(10) ^{13}C NMR (CD_3OD): δ 204.29, 199.32, 180.39, 99.24, 88.25, 36.16, 31.22, 28.59, 25.54.
(11) When treated with $NaBH_4$ for a few minutes, **1** was rapidly converted to **3**. Similarly **2** was smoothly reduced to **3**.
(12) For example, see: Mallams, A. K.; Puar, M. S.; Rossman, R. R.; McPhail, A. T.; MacFarlane, R. D.; Stephen, R. L. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1497. Kobinata, K.; Uramoto, M.; Mizuno, T.; Isono, K. *J. Antibiot.* **1980**, *33*, 244.

Binding of Thallium(I) to a [3Fe-4S] Cluster: Evidence for Rapid and Reversible Formation of $[Tl3Fe\text{-}4S]^{2+}$ and $[Tl3Fe\text{-}4S]^{1+}$ Centers in a Ferredoxin

Julea N. Butt, Artur Sucheta, and Fraser A. Armstrong*

Department of Chemistry, University of California Irvine, California 92717

Jacques Breton and Andrew J. Thomson

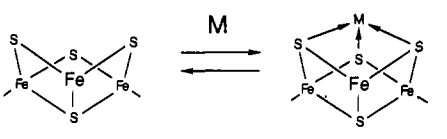
School of Chemical Sciences, University of East Anglia Norwich NR4 7TJ, England

E. Claude Hatchikian

Laboratoire de Chimie Bacterienne CNRS, PB 71, 13277 Marseille, France

Received May 28, 1991

A number of proteins contain [3Fe-4S] centers that bind certain metal ions reversibly to form cubane-like clusters of the type [M3Fe-4S].[1-7]



0002-7863/91/1513-8948$02.50/0

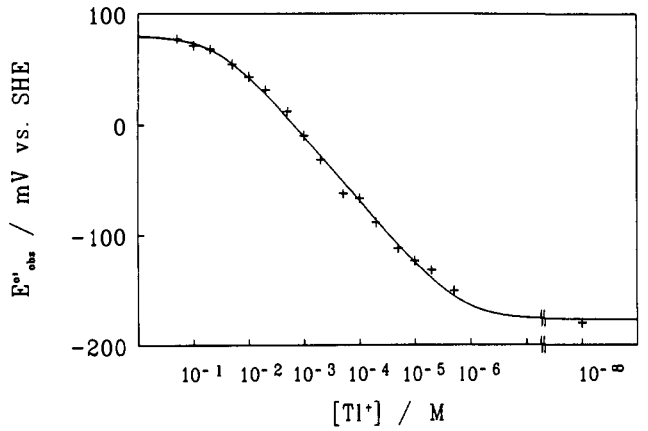


Figure 1. Graph of observed reduction potentials (see footnote 10) against concentration of Tl^+. The curve is the computed nonlinear regression fit to the equation $E^{\circ\prime}_{obs} = E^{\circ\prime} + (2.303mRT/F) \log \{(1 + [Tl^+]/K_d^{red})/(1 + [Tl^+]/K_d^{ox})\}$ where m is the number of Tl^+ ions bound. Parameters are as follows: $K_d^{red} = 1.5 \pm 1.0$ μM; $K_d^{ox} = 34 \pm 10$ mM; $d(E^{\circ\prime}_{obs})/d(\log [Tl^+])$ (maximum) = 59.2 ± 2.0 mV (thus m = 1.06); $E^{\circ\prime} = -177 \pm 5$ mV. The value $E^{\circ\prime}_{Tl} = +81 \pm 10$ mV was calculated from the equation $E^{\circ\prime}_{Tl} = E^{\circ\prime} + (2.303mRT/F) \log (K_d^{ox}/K_d^{red})$.

The reaction with M = Fe occurs during in vitro activation of aconitase[1] and may be the basis for regulation of Fe levels in eukaryotic cells by the iron-responsive element binding protein which bears considerable sequence homology with aconitase.[8] The metal ion specificity is interesting, particularly since the ubiquity of [4Fe-4S] clusters has led to a tacit assumption (possibly mistaken) that there is always a clear preference for M = Fe. Clusters with M = Co, Zn, Cd, or Ni have been generated in vitro for different proteins[5,6] and we have recently shown[7] that the $[3Fe\text{-}4S]^0$ cluster in 7Fe ferredoxin III from *Desulfovibrio africanus* has a higher affinity for Zn^{2+} or Cd^{2+} than for Fe^{2+}. These transformations have had in common the observation that a *divalent* metal ion reacts with the *reduced* 3Fe cluster $[3Fe\text{-}4S]^0$. Here we report rapid and reversible interaction of both 0 and 1+ oxidation levels of the [3Fe-4S] cluster of Fd III with the monovalent metal ion Tl^+.

We used the very sensitive technique described previously,[7,9] that is, the voltammetry of coadsorbed protein/aminocyclitol films at edge-oriented pyrolytic graphite electrodes. This enables time-domain monitoring of active-site transformations through observation of voltammetric signals assigned to specific cluster states by EPR and MCD. Because so few protein molecules are addressed, the technique (besides being extremely economical on sample) is ideal for examining reactions with very dilute reagents (concentrations $\ll 10^{-4}$ M). For these studies, after preequilibrating a neomycin film of the 7Fe protein in electrolyte containing EGTA, the electrode was transferred to solutions (temperature 7 °C) containing Tl^+ (as the acetate salt, pH 7, I = 0.5 (Na acetate)).

As shown in Figure 1, the observed reduction potential $E^{\circ\prime}_{obs}$ for the $[3Fe\text{-}4S]^{1+/0}$ couple varies with Tl^+ concentration.[10] Concurrently, the indigenous $[4Fe\text{-}4S]^{2+/1+}$ couple is unaffected.[11] No shift of $E^{\circ\prime}_{obs}$ occurs if 0.1 M K^+ (radius 138 pm) or 0.1 M Rb^+ (152 pm) is substituted for Tl^+ (150 pm).[12] Data conform well to a model, Scheme I, in which there is rapid, reversible interaction of Tl^+ at both "0" ($K_d = 1.5 \pm 1.0$ μM) and "1+" forms ($K_d = 34 \pm 10$ mM) of the 3Fe cluster. Thallium inhibits coordination of Fe^{2+} at the $[3Fe\text{-}4S]^0$ core, as shown by comparing the voltammetry observed upon transfer to electrolyte containing 30 μM Fe^{2+} with that observed for a 30/5 μM mixture of Fe^{2+} and Tl^+. In the latter case, the signal due to the transformed $[4Fe\text{-}4S]^{2+/1+}$ couple (K_d for $[4Fe\text{-}4S]^{2+}$ = 30 ± 15 μM)[7] was greatly attenuated.

Such results suggest that Tl^+ coordinates at the 3Fe core. Indeed, the tri-μ_2-sulfido cluster face is the only polarizable ligand group on the protein likely to discriminate so effectively in favor of Tl^+ (electronic configuration $d^{10}s^2$) against K^+ or Rb^+. This proposal was supported by EPR measurements on a sample of fully oxidized protein (174 μM Fd III in 0.1 M Hepes, pH 7.5) to which thallium(I) acetate had been added to give $[Tl^+]$ = 133 mM. The characteristic g = 2.01 signal of $[3Fe\text{-}4S]^{1+}$ was replaced by a rhombic spectrum[13] with g values 2.042, 1.993, and 1.954. That the situation in solution may also be described as a reversible system at equilibrium was shown by deconvolution of EPR spectra observed at lower Tl^+ levels. These spectra were a superposition of signals from the new species and from $[3Fe\text{-}4S]^{1+}$, in ratios consistent with those expected from the K_d value derived from the film experiments.[14]

The reduction potential of the $[Tl3Fe\text{-}4S]^{2+/1+}$ couple is 81 mV, an increase of ca. 0.25 V over the value[15] for $[3Fe\text{-}4S]^{1+/0}$. Thus

(1) Beinert, H.; Kennedy, M. C. *Eur. J. Biochem.* **1989**, *186*, 5. Beinert, H. *FASEB J.* **1990**, *4*, 2483.

(2) Moura, J. J. G.; Moura, I.; Kent, T. A.; Lipscomb, J. D.; Huynh, B.-H.; LeGall, J.; Xavier, A. V.; Munck, E. *J. Biol. Chem.* **1982**, *257*, 6259. Conover, R. C.; Kowal, A. T.; Fu, W.; Park, J.-B.; Aono, S.; Adams, M. W. W.; Johnson, M. K. *J. Biol. Chem.* **1990**, *265*, 8533.

(3) George, S. J.; Armstrong, F. A.; Hatchikian, C. C.; Thomson, A. J. *Biochem. J.* **1989**, *264*, 275.

(4) Robbins, A. H.; Stout, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3639.

(5) Moura, I.; Moura, J. J. G.; Muenck, E.; Papaefthymiou, V.; LeGall, J. *J. Am. Chem. Soc.* **1986**, *108*, 349. Surerus, K. K.; Muenck, E.; Moura, I.; Moura, J. J. G.; LeGall, J. *J. Am. Chem. Soc.* **1987**, *109*, 3805.

(6) Conover, R. C.; Park, J.-B.; Adams, M. W. W.; Johnson, M. K. *J. Am. Chem. Soc.* **1990**, *112*, 4562.

(7) Butt, J. N.; Armstrong, F. A.; Breton, J.; George, S. J.; Thomson, A. J.; Hatchikian, E. C. *J. Am. Chem. Soc.* **1991**, *113*, 6663.

(8) Rouault, T. A.; Stout, C. D.; Kaptain, S.; Harford, J. B.; Klausner, R. D. *Cell* **1991**, *64*, 881.

(9) Armstrong, F. A.; Butt, J. N.; George, S. J.; Hatchikian, E. C.; Thomson, A. J. *FEBS Lett.* **1989**, *259*, 15.

(10) Observed reduction potentials $E^{\circ\prime}_{obs}$ were measured from the average of peak positions for scans (typically at a rate of 190 mV/s) in the directions of decreasing and increasing potential. In previous work (refs 7 and 9), we have found that $E^{\circ\prime}_{obs}$ values determined for the redox couples of Fd III in aminocyclitol films are very similar to those for the protein in bulk solution.

(11) Reduction of Tl^+ to Tl^0 severely restricted our voltammetry at low potentials. Such excursions produced a $Tl^{0\rightarrow 1+}$ stripping wave on the return scan. Even so, under conditions of low $[Tl^+]$, we observed that the appearance and position of the indigenous $[4Fe\text{-}4S]^{2+/1+}$ couple (B′ of ref 7) were unchanged when compared to scans over the same limited range in the absence of Tl^+. Reduction of Tl^+ also prevented examination of simultaneous changes associated with couple C′ (see ref 7).

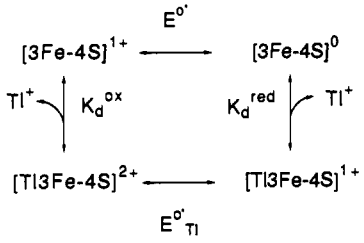
(12) Greenwood, N. N.; Earnshaw, A. *Chemistry of the Elements*; Pergamon Press: New York, 1984.

(13) This signal broadens as the temperature is raised, being too weak to be detected at 30 K. No hyperfine splitting due to interaction of the electron spin with the Tl nucleus could be detected. This may be due to the low nuclear magnetic moments of ^{203}Tl ($I_N = {}^1/_2$) and ^{205}Tl ($I_N = {}^1/_2$), namely, +1.6 nuclear magnetons. No other signals apart from an exceedingly weak feature at g = 4.3, assigned to adventitious Fe(III), could be detected over the full sweep range of the EPR spectrometer. The spectrum of a sample of the reduced cluster, $[3Fe\text{-}4S]^0$, in the presence of an equimolar amount of thallium(I) acetate showed no signals. The magnetic properties of this species remain to be investigated further.

(14) Deconvolution of the EPR spectra of two samples prepared with lower Tl^+ concentration enabled the following relative fractions $[3Fe\text{-}4S]^{1+}$/$[Tl3Fe\text{-}4S]^{2+}$ to be determined: for $[Tl^+]$ = 19.5 mM, ratio = 2.7; and for $[Tl^+]$ = 100 mM, ratio = 0.09. The K_d values estimated from these ratios are 54 and 10 mM, respectively, giving an average value K_d = 32 mM.

(15) The more negative $E^{\circ\prime}$ value for the $[3Fe\text{-}4S]^{1+/0}$ couple observed (at zero $[Tl^+]$) in these studies compared to previous work (−140 mV, ref 7 and 9) is due to ionic strength effects rather than specific involvement of acetate ion at high concentration. Identical voltammetry for a film of 7Fe Fd III was observed upon transfer to electrolyte containing no acetate, but with I = 0.5 M (NaCl), pH 7.0 (Hepes). We used 0.5 M acetate as electrolyte in order to obtain high solubility of Tl^+ for defining the upper limiting curvature in the plot of $E^{\circ\prime}_{obs}$ vs log $[Tl^+]$. Experiments on Tl^+ binding using 0.1 M $NaNO_3$, pH 7 (Hepes), as the supporting electrolyte showed behavior similar to that of Figure 1 with the exception of the potential shift mentioned above. Thus acetate and Cl^- are unlikely to be exogenous ligands to thallium.

Scheme I



Tl^+ effectively stabilizes the "0" level. Coordination of Tl^+ to the oxidized cluster is weak, but nevertheless represents the first reported interaction of a metal ion other than Fe^{2+} with the less electron rich $[3Fe\text{-}4S]^{1+}$ species.[16] In the amino acid sequence that forms the binding domain for the [3Fe-4S] cluster, a cysteine that would normally provide[17] the fourth ligand for [4Fe-4S] centers is replaced by aspartate.[3,18] Thus carboxylate and/or H_2O (OH^-) are likely noncluster ligand(s) to thallium.[15]

Because of the similarity of Tl^+ and K^+ in charge and size, Tl^+ has been used to probe K^+ binding sites in enzymes[19] and ion channels.[20] Here, a different type of interaction is evident, one showing the preference of Tl^+ for polarizable ligands. The affinity of Tl^+ for $[3Fe\text{-}4S]^0$ is much higher than values reported for K^+ binding sites (for which K_d is typically in the millimolar range[19]) and implicates the cluster as a possible biological target for this toxic element.[21] There is an interesting similarity with the crown thioether complex $[Tl([9]aneS_3)]^{1+}$ in which Tl(I) is coordinated facially with an average Tl-S distance of 3.1 Å.[22] The [3Fe-4S] core also provides a tripodal S donor system, but one for which the propensity for metal ion coordination can be modulated by the core oxidation level. Observation of near-ideal wave shapes[23] throughout the concentration range 10^{-5}–0.5 M Tl^+ at scan rates up to 470 mV s^{-1} shows that Tl^+ "on" and "off" rates are fast. Thus the metal binding site at [3Fe-4S] in Fd III is probably exposed to solvent, and the protein offers little resistance to the transformation. Because of the possibility of interference with normal biochemical function, it is of interest to determine how general this reactivity is.

Acknowledgment. This work was supported by the University of California, by grants from NATO (CRG 900302) and from the Molecular Recognition Initiative of the SERC of the UK, and by an Exxon Education Foundation Award (F.A.A.).

(16) The core cluster $[4Fe\text{-}4S]^{3+}$, which represents the product of the reaction between Fe^{2+} and the oxidized cluster $[3Fe\text{-}4S]^{1+}$, is known in proteins called high-potential iron proteins, HiPIP (Carter, C. W., Jr.; Kraut, J.; Freer, S. T.; Alden, R. A. *J. Biol. Chem.* **1974**, *249*, 6339) and also a synthetic model compound $[Fe_4S_4(S\text{-}2,4,6\text{-}(i\text{-}Pr)_3C_6H_2)_4](Bu_4N)$ (O'Sullivan, T.; Millar, M. M. *J. Am. Chem. Soc.* **1985**, *107*, 4096).

(17) Fukuyama, K.; Nagahara, Y.; Tsukihara, T.; Katsube, Y.; Hase, T.; Matsubara, H. *J. Mol. Biol.* **1988**, *199*, 183. Otaka, E.; Ooi, T. *J. Mol. Evol.* **1987**, *26*, 257.

(18) Bovier-Lapierre, G.; Bruschi, M.; Bonicel, J.; Hatchikian, E. C. *Biochim. Biophys. Acta* **1987**, *913*, 20.

(19) Kayne, F. J. *Arch. Biochem. Biophys.* **1971**, *143*, 232. Markham, G. D.; Hafner, E. W.; Tabor, C. W.; Tabor, H. *J. Biol. Chem.* **1980**, *255*, 9082. Bostain, K. A.; Betts, G. F.; Man, W. K.; Hughes, M. N. *Biochem. J.* **1982**, *207*, 73. Markham, G. D.; Leyh, T. S. *J. Am. Chem. Soc.* **1987**, *109*, 599. Lord, K. A.; Reed, G. H. *Inorg. Chem.* **1987**, *26*, 1464.

(20) Miller, I. R. *Biochim. Biophys. Acta* **1985**, *817*, 199. Nelson, A. *J. Electroanal. Chem.* **1991**, *303*, 221.

(21) Douglas, K. T.; Bunni, M. A.; Baindur, S. R. *Int. J. Biochem.* **1990**, *22*, 429.

(22) Blake, A. J.; Greig, J. A.; Schroeder, M. *J. Chem. Soc., Dalton Trans.* **1991**, 529.

(23) Half-height widths were 100–105 mV; see: Laviron, E. *J. Electroanal. Chem.* **1974**, *52*, 355, 395. At Tl^+ concentrations below 5 μM, we observed that the voltammetric signals became distorted by asymmetric broadening. The reason for this is unclear but may be a combination of $[Tl^+]$ levels being lower than needed for saturation of the binding sites, combined with the decreased "on" rate. The effect could be decreased by use of a rotating disk electrode (minimizing mass transport limitations) and by use of slow scan rates. Even so, voltammograms for this low concentration region were not included in the data analysis and were unnecessary because of the high confidence in $E^{\circ\prime}([Tl]^+ = 0)$. Further investigations of this phenomenon are under way.

Stereoselective Zirconium-Catalyzed Ethylmagnesation of Homoallylic Alcohols and Ethers. The Influence of Internal Lewis Bases on Substrate Reactivity

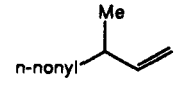
Amir H. Hoveyda,* Zhongmin Xu, James P. Morken, and Ahmad F. Houri

Department of Chemistry, Boston College
Chestnut Hill, Massachusetts 02167

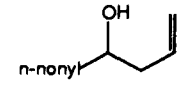
Received July 11, 1991

We recently reported a zirconium-catalyzed carbomagnesation that effects the stereoselective addition of EtMgCl to unactivated alkenes.[1] Reactions of allylic alcohols and ethers proceed with a complementary sense of stereoinduction. The reversal of stereoselectivity was attributed to the association of the magnesium alkoxide (and not ethers) with the zirconium reagent. Herein we report on the ethylmagnesation of acyclic and cyclic homoallylic alcohols and ethers.

As is illustrated in Table I, treatment of the anti homoallylic alcohol **1a** with 4 equiv of EtMgCl and 5 mol % Cp_2ZrCl_2 in Et_2O (25 °C, 12 h), followed by the addition of $B(OMe)_3/H_2O_2$ (−78 °C), provides **2a** in 75% isolated yield with ≥99:1 selectivity.[2] Ethylmagnesations of **1b** and **1c** proceed with the same sense of stereoinduction as is observed with **1a**. Several conclusions can be derived from the study of the carbometalations of **1a–c**: (1) Binding from the homoallylic position is more effective than from the allylic site. The adverse influence of THF on the stereochemical outcome of the reactions of homoallylic metal alkoxides is less pronounced than on that of the allylic systems.[1] The uniform sense of stereoinduction in reactions of **1a–c** implies that, in contrast to allylic ethers, homoallylic ethers may bind to the transition metal and direct the course of the reaction. (2) Internal chelation leads to selectivity. With a more effective internal Lewis base, higher stereoselectivities are observed (see **1a** and **1c**); THF does not compete with a strong ligating group, but alters the binding of less efficient internal ligands (OMEM, **1c**) to inflict a diminution in stereocontrol. Without internal coordination, no stereoselectivity can be achieved: ethylmagnesation of **4** provides a 1:1 diastereomeric mixture of products. (3) Heteroatom–metal coordination leads to enhanced reactivity. When such coordination is altered, either because of an inferior Lewis base (compare **1a** and **1c**, Table I) or due to the presence of a competing ligating solvent (THF), reaction efficiency is seriously reduced. With both an inferior Lewis base and a coordinating solvent, no reaction is observed (**1c** in THF, Table I). In accord with this trend, whereas carbometalation of **1a** occurs in 75% yield, that of **4** proceeds to only 15% conversion (90% mass balance).



4

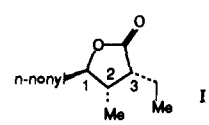


8

Ethylmagnesation of syn homoallylic alcohol **5a** (Table II) proceeds less efficiently and selectively (55%, 85:15) than that of the corresponding anti isomer **1a** (75%, ≥99:1). Unlike the

(1) Hoveyda, A. H.; Xu, Z. *J. Am. Chem. Soc.* **1991**, *113*, 5079–5080. For related studies, see: (a) Takahashi, T.; Seki, T.; Nitto, Y.; Saburi, M.; Rousset, C. J.; Negishi, E. *J. Am. Chem. Soc.* **1991**, *113*, 6266–6268. (b) Knight, K. S.; Waymouth, R. M. *J. Am. Chem. Soc.* **1991**, *113*, 6268–6270.

(2) All compounds reported herein gave ^1H NMR, ^{13}C NMR, IR, and combustion analysis data consistent with the structure given. The stereochemical identity of **2a** was determined through decoupling and NOE experiments on the derived lactone I (Pt/O_2); enhancements were observed between H_2 and H_3, and H_1 and H_2, and none was observed between H_1 and H_3. Reaction of I with DBU/MeOH led to the formation of the all-equatorial, anti,anti isomer (100%); further supporting NMR studies were performed on the latter compound. See the supplementary materials for details.



I

0002-7863/91/1513-8950$02.50/0