Table I. Some Spectral Data of **1**

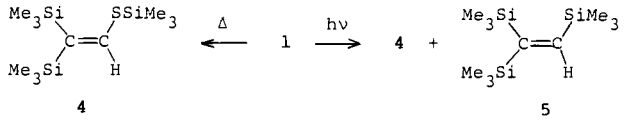
electronic spectrum/λ_{max}/nm (ϵ)		^{1}H NMR, δ		^{13}C NMR,	^{29}Si NMR,	
hexane	CH_3CN	$CDCl_3$	C_6D_6	δ, $CDCl_3$	δ, $CDCl_3$	IR, cm^{-1}, KBr
518 (15)	503 (14)	0.26 (s, 27 H)	0.16 (s, 27 H)	2.6	-0.22	1120 (C=S)
272 (9940)	277 (8720)	11.45 (s, 1 H, C*H*S)	11.45 (s, 1 H, C*H*S)	59.0		
212 (4320)	211 (5330)			248.2 (*C*HS)		

Table II. Thermodynamic Parameters of the Thermolysis of **1** and **5**[a]

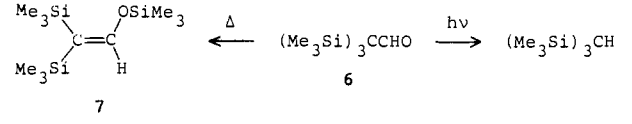
entry	compound	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}$/eu	$\tau_{1/2}$ (70 °C)/h
1	**1**	28.0 ± 0.7	0.4 ± 2.1	16
2	**6**	24.2 ± 1.6	-6.1 ± 4.9	1.7
3[b]	$R_3SiCH_2C(=O)R'$	26-33[c]	-4.2 to -16.9	

[a] The thermolyses were carried out in toluene-d_8 at 70-90 °C for **1** and at 56-70 °C for **6**. [b] Reference 17. [c] E_a values.

vinyl sulfide **4** quantitatively.[15] It is noteworthy that **1** does not undergo oligomerization but isomerization upon heating. In contrast to the thermolysis, the photolysis of **1** (medium-pressure Hg lamp, benzene, 5 °C, 17 h) afforded **5** (33%)[18] in addition to **4** (66%).



For comparison, the corresponding aldehyde **6**,[15] prepared from **2** and ethyl formate, was also subjected to the thermolysis and photolysis under similar conditions to give silyl enol ether **7**[19] (100%) and tris(trimethylsilyl)methane[12] (90%), respectively.



The difference in photochemical behavior between **1** and **6** is noteworthy. The formation of **5** from **1** is especially interesting since this type of reaction, i.e., 1,2-shift of a group from the α-position to the thiocarbonyl carbon with a concomitant loss of sulfur, is a new mode of photoreaction for thiocarbonyl compounds. This reaction most likely proceeds as shown in Scheme I. The vinyl sulfide **4** cannot be an intermediate of **5** since **4** is inactive under the reaction conditions.

Interestingly, **1** is thermally more stable than the corresponding aldehyde **6**. The thermolyses of **1** and **6** obey first-order kinetics, and the half-lives of **1** and **6** in toluene-d_8 at 70 °C are 16 and 1.7 h, respectively. The thermodynamic parameters for these reactions are shown in Table II along with those obtained for α-silyl ketones by Brook,[17] which are in good agreement with those for **6**. Comparison of the parameters for **1** and **6** shows that the stability of **1** compared with **6** results mainly from an enthalpic factor, i.e., the much smaller bond energy of Si-S than that of Si-O.

The thioaldehyde **1** was reduced with sodium borohydride to give the corresponding thiol, $(Me_3Si)_3CCH_2SH$ (**8**),[15] quantitatively.

When **1** was treated with methyllithium and *tert*-butyllithium, the olefin **5** was formed in 79% and 34% yields, respectively, although in the latter reaction the thiol **8** (35%) and $(Me_3Si)_3CCH_2S$-*t*-Bu (**9**) (10%) were also produced. The corresponding Grignard reagents react with **1** in a similar manner but with a much slower rate.

Acknowledgment. We thank Shin-etsu Chemical Co. Ltd. for the gift of chlorosilanes. The financial support of the Ministry of Education, Science and Culture of Japan is gratefully acknowledged.

Supplementary Material Available: Spectral data and exact mass analyses for new compounds (2 pages). Ordering information is given on any current masthead page.

(17) Brook, A. G. *Acc. Chem. Res.* **1974**, *7*, 77.
(18) Bock, H.; Seidl, H. *J. Organomet. Chem.* **1968**, *13*, 87.
(19) This compound has been reported but not obtained in the pure form: Dunoguès, J.; Jousseaume, E.; Pillot, J.-P.; Calas, R. *J. Organomet. Chem.* **1973**, *52*, C11. See ref 15.

Dehydrogenase Inactivation by an Enzyme-Generated Acetylenic Ketone: Identification of a Lysyl Enaminone by ^{13}C NMR

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Mechanism-based inactivation[1] by enzyme-generated acetylenic ketones[2] has been assumed to occur by Michael addition of an enzymic nucleophile to these potent electrophiles. No structural information has been presented, however, that supports the presumed inactivation mechanism. Recently, we have extended our studies of acetylenic steroid inhibitors of estrogen biosynthesis to human placental estradiol dehydrogenase (E_2-HSD),[2a] an enzyme that catalyzes the interconversion of estrone and estradiol. We now present evidence that Michael addition of the ϵ-amino group of a lysine residue accompanies inactivation of E_2-HSD by enzyme-generated 3-hydroxy-14,15-secoestra-1,3,5(10)-trien-15-yn-17-one[3] (**2**) (Scheme I).

Incubation of 40 μM [15,16-$^{13}C_2$]-14,15-secoestra-1,3,5-(10)-trien-15-yne-3,17β-diol[4] ([$^{13}C_2$]-**1**) with 100 μM NAD^+ and E_2-HSD[5] for 18 h at pH 9.2 produced >90% loss of initial enzymatic activity.[6] The ^{13}C NMR spectrum of the inactivated enzyme did not reveal any major features not present in the spectrum of enzyme inactivated with natural-abundance steroid (Figure 1a,b). We attribute our inability to observe the steroidal ^{13}C resonances to the large molecular weight of the enzyme (M_r = 68 000) and its tendency to form aggregates in the absence of glycerol,[7] affording relatively long correlation times and line

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(4) Prepared as in ref 3 except using [$^{13}C_2$]HC≡CLi in the last step (from 99% [$^{13}C_2$]HC≡CH and *n*-BuLi in THF/-78 °C[15]).
(5) Purified from human term placenta by the method of Murdock et al. (Murdock, G. L.; Chin, C.-C.; Warren, J. C. *Biochemistry* **1986**, *25*, 641-646) except that a second ammonium sulfate precipitation was added after the heat step and that Reactive Red 120-Agarose was used in place of Reactive Blue 2-Agarose (Sigma Chemical Co., St. Louis, MO).
(6) Inactivated enzyme was concentrated by ultrafiltration and dialyzed vs. four changes of 10 mM KPO_4, pH 7.0, containing 1 g/L bovine serum albumin in the first two changes, both at 4 °C. The dialyzed protein was filtered, further concentrated to ~1.5 mL by suction ultrafiltration, and diluted with D_2O to ~1.7 mL in a 10-mm NMR tube.

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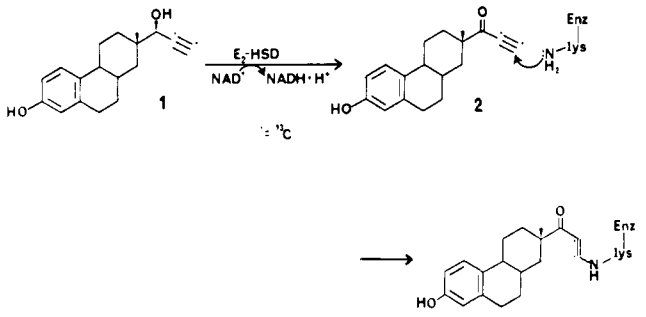
Scheme I



broadening due principally to short T_2 values.[8]

The signals in these spectra (Figure 1a,b) probably derive principally from mobile residues on the surface of the enzyme. Our inability to observe steroidal resonances in the intact protein indicates that the steroid is rigidly bound and not on the periphery of the protein, consistent with our previous results that suggest active-site-directed inactivation.[2a] Digestion of the inactivated enzyme samples with pronase[9] generated flocculent precipitates that contained the majority of the steroid. Several resonances that did not report in the spectrum of intact protein, such as the aromatic amino acids (125–135 ppm), appeared after pronase digestion. Two broad features with fine structure were identified as steroidal resonances due to their absence in the natural abundance sample (Figure 1c–f).

To elucidate the structure of this enzyme–steroid adduct, the chemical shifts of the enriched carbon atoms in the adduct were compared with those of a series of model compounds.[10] The chemical shifts of only the enaminone (Figure 1g) correspond to those found in the steroidal adduct, indicating that we detect one enzyme nucleophile that forms a Michael adduct with the enzyme-generated acetylenic ketone; furthermore, that enzymic nucleophile is a lysine residue. Since the chemical shift of the C_β resonance of the steroidal adduct corresponds closely to the C_β resonance of the (*E*)-enaminone model adduct (Figure 1g, 154 ppm), we propose that the steroid–peptide adduct exists primarily as the *E* isomer.

The rather broad spectral features despite pronase digestion may be due to the remarkably poor solubility of the steroid-bound peptide fragment.[11] The width of the enriched steroidal resonances suggests that the steroid resides in an environment of restricted mobility, perhaps bound to SDS micelles. In addition, our model lysine adduct exists, in HOD, as a mixture of *E* and *Z* isomers in slow exchange, and the C_α resonance broadens reversibly (due to proton exchange)[12] upon deprotonation of the enaminone (Figure 1g). More rapid proton exchange rates and/or a lower enamine pK_a in the peptide fragment could also contribute to the observed line broadening. Nevertheless, the chemical shift data presented here and in our model study enable us to identify unambiguously the structure of this enzyme–steroid adduct as a lysyl enaminone. Further studies are in progress to determine if other undetected (immobilized) adducts exist.

Histidine[13] and cysteine[13c] residues have been modified by bromo- and iodoacetoxy (or acetamido) steroidal affinity labels, but these inactivations were conducted at pH 6.3[13a,b] or 7.2.[13c]

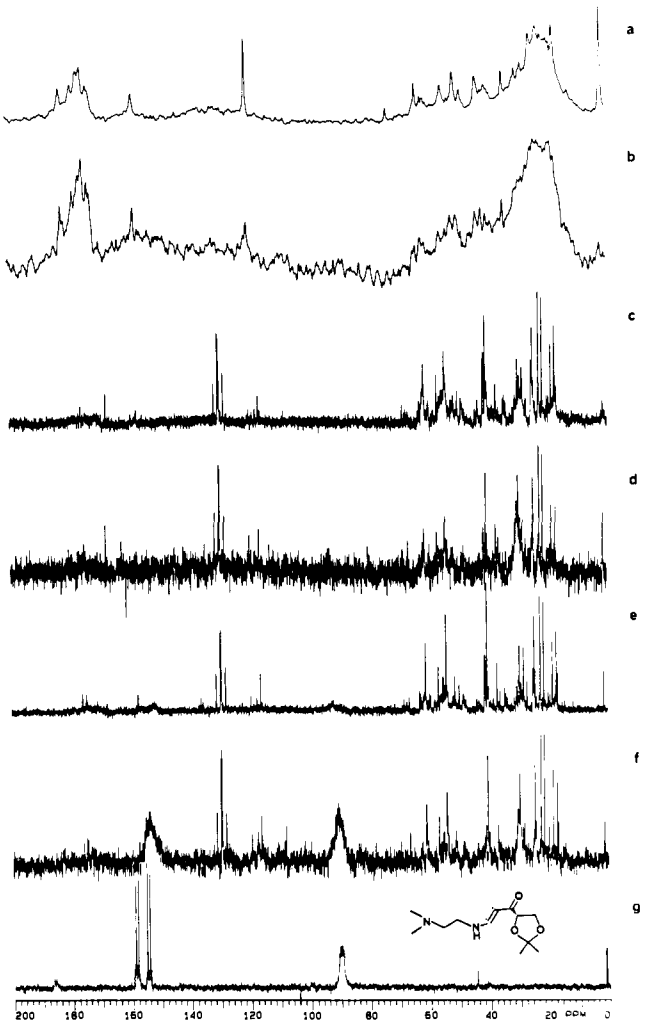


Figure 1. $^{13}C\{^1H\}$ NMR spectra of (a) 100 mg of E_2-HSD inactivated with natural-abundance alcohol **1** and NAD^+ (all samples except (b) contained 10 μL of CH_3CN, 1.3 ppm internal reference—118 ppm C≡N signal not always observed), 10-μs pulse, 0.9-s acquisition (no delay), 39 024 scans, 35-Hz exponential line broadening, and double-precision data processing; (b) 143 mg of E_2-HSD inactivated by incubation with [$^{13}C_2$] alcohol **1** and NAD^+, 2 μL of CH_3CN added, as in (a) except 38 402 scans; (c) supernatant and (d) pellet from pronase digest of sample from (a), 20-μs pulse, 1.0-s acquisition, 3-Hz line broadening, 2000 scans; (e) supernatant and (f) pellet from pronase digest of sample from (b), as in (c) and (d) except 25-μs pulse, 1000 scans; (g) mixture of (*E*) [$\delta(C_\beta)$ 154.4] and (*Z*)-enaminones [$\delta(C_\beta)$ 158.2] (HOD, pH >11), 10-μs pulse, 1.0-s acquisition, 3-Hz line broadening, 515 scans. Spectra were recorded at ambient temperature at 75 MHz by using a Varian XL-300 spectrometer equipped with a 10-mm probe (except (g), 5 mm). WALTZ-16 decoupling was employed continuously in all samples.

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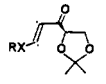
(9) Samples corresponding to spectra (a) and (b) were incubated at 25 °C with 2 mg of pronase for 19 or 16 h in 9 or 14 mL of 10 mM KPO_4, pH 7.0, respectively. SDS-PAGE confirmed that no fragments of $M_r > 10\,000$ remained. After centrifugation (30 min, 40 000 rpm), the supernatants were lyophilized and dissolved in 1.6 mL of 20% aqueous D_2O or 1.7 mL of 12% D_2O, respectively. The pellet from (b) was resuspended in 400 μL of $NaPO_4$, pH 8.0, 1% sodium dodecyl sulfate (SDS); diluted with 3 mL 10 mM $NaPO_4$, pH 8.0; incubated at 25 °C for 17 h with 2 mg of pronase; lyophilized; and resuspended in 1.7 mL of 12% aqueous D_2O. The pellet from (a) was resuspended in 300 μL of 10 mM $NaPO_4$, pH 7.0, 1% SDS, and diluted with 800 μL of 1:1 10 mM $NaPO_4$, pH 7.0/D_2O.

(10) A series of adducts were prepared:



where · = ^{13}C; R = $(CH_3)_2NC_2H_4$ and X = O, PhO, S, NH, imidazole, NHC(=NH)NH or R = C_2H_5 and X = COO. Chemical shifts and coupling constants for the enriched carbon atoms in both $CDCl_3$ and 20 mM KPO_4 (pH 7.0, 20% D_2O) were tabulated. Only in the enaminone (X = NH) did the C_α resonance occur at ~ 90 ppm; in all other adducts, the chemical shifts were ≥100 ppm. Electron donation from nitrogen probably contributes to the upfield position of C_α in the enaminone. The stereochemistry about the C=C bonds was ascertained from the ^1H NMR spectra of natural abundance adducts. Details concerning the model compounds will be published elsewhere.

(11) Addition of CH_3CN or Me_2SO did not solubilize the sample. Also, we cannot rule out that heterogeneous protein digestion contributes to line broadening through chemical shift heterogeneity.

(12) In the model adduct, C_α–H is exchanged in alkaline D_2O.

(13) (a) Murdock, G. L.; Chin, C.-C.; Offord, R. E.; Bradshaw, R. A.; Warren, J. C. *J. Biol. Chem.* **1983**, *258*, 11460–11464. (b) Murdock, G. L.; Chin, C.-C.; Warren, J. C. *Biochemistry* **1986**, *25*, 641–646. (c) Pons, M.; Nicolas, J.-C.; Boussioux, A.-M.; Descomps, B.; Crastes de Paulet, A. *Eur. J. Biochem.* **1976**, *68*, 385–394.

Perhaps the higher pH at which our incubations were performed[14] deprotonates this lysine ($pK_a \sim 9$–10) and renders it sufficiently nucleophilic to compete with other nucleophilic residues in the active site. To our knowledge, this is the first report of a lysine residue in E_2-HSD modified by an active-site-directed alkylating agent.

Acknowledgment. This work was supported by NIH Grants CA-23582, Research Career Development Award CA-00829 (D.F.C.), and National Research Service Award GM-07805 (R.J.A.). Assistance was provided by the Washington University High Resolution NMR Facility, supported in part by NIH 1 S10 RR00204 and a gift from Monsanto Co. We thank Mary Ann Sullivan, R.N., and her staff at St. John's Mercy Medical Center, St. Louis; Dr. Gene Z. Chiao; and Patrick C. McMullan for assistance in obtaining human term placenta.

(14) High pH was necessary to drive the equilibrium toward oxidation of alcohol to ketone (see Scheme I and ref 2a).
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Expansion of the Molybdenum Coordination Sphere in Dinuclear Tetrasulfur-Bridged Complexes with the $Cp_2Mo_2S_4$ Core

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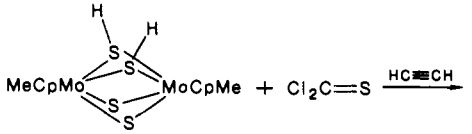
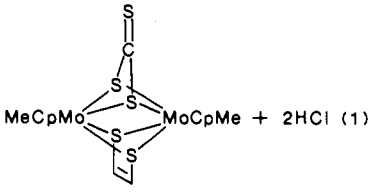
The reaction of the dinuclear hydrosulfido-bridged molybdenum complex with thiophosgene has been reported to yield a Mo(III) derivative with a μ-η^2-trithiocarbonate ligand (eq 1).[1] Although





I

several mononuclear and dinuclear trithiocarbonate complexes have been prepared,[2-7] we have not found previous reports of the η^2-bridging mode for this ligand. We have, therefore, begun a study of the reactivity of the thiocarbonyl functional group in complex I. We report here an interesting result which reveals reactivity characteristics of the molybdenum ions in these systems in which sulfur ligand reactivity normally dominates the chemistry.[8-10]

Unlike the thiocarbonyl functional group in organic molecules, the C=S bond of the trithiocarbonate ligand is resistant to hydrolysis. Complex I was recovered nearly quantitatively from an aqueous/THF solvent mixture which had been refluxed for 3 weeks. The addition of a small amount of trifluoroacetic acid did not alter these results. However, when complex I was reacted with 2–3 equiv of methyl iodide at room temperature in THF, a reaction did occur to form an 80% yield of an orange product.[11] The complex was formulated as the salt $[(MeCpMo)_2(SC_2H_2S)S_2CSMe]I$ (II). Elemental analyses are consistent with the proposed composition.[11] The room temperature proton NMR spectrum in $CDCl_3$ shows sharp resonances at 2.87 and 7.2 ppm which are consistent with a SMe group and the alkenedithiolate ligand, respectively. However, the methylcyclopentadienyl resonances at 6.06 and 2.2 ppm are broad singlets at room temperature. A variable-temperature NMR study confirms that there is a fluxional process occurring in solution. At -54 °C, two singlets are observed for the Cp ring protons at 6.41 and 5.66 ppm and for the Cp–Me protons at 2.49 and 1.89 ppm. As the temperature is increased, these resonances broaden with coalescence for the Cp–Me resonances occurring at 0 °C and for the Cp signals at 6 °C. On the basis of these coalescence temperatures, the free energy of activation for the fluxional process is calculated to be 56 kJ/mol.[12] The ^{13}C NMR spectrum also shows a variation with temperature.[13]

The process which results in inequivalent cyclopentadienyl resonances at low temperatures was proposed to involve the interaction of the methylated ligand with a molybdenum ion in the cation. The nature of this interaction has been established by an X-ray diffraction study. The complex crystallized in space group $P21/c$ with four molecules per unit cell.[14] A perspective drawing of the molecule is shown in Figure 1. The structure consists of discrete cations and anions. The structure of the cationic dinuclear molybdenum complex confirms that the terminal sulfur atom of the trithiocarbonate ligand has been methylated. The C–S bond distances around this terminal sulfur atom lie within the normal range for single bonds. The ligand is coordinated to Mo_1 through the other two sulfur atoms S_2 and S_3 and to Mo_2 through sulfur atoms 2 and 3 and the carbon atom (C_1) of the ligand. The Mo–C distance of 2.195 (6) Å is typical of a single bond between these atoms.[15] The CS_3 portion of the ligand retains its planarity, and by chelating to Mo_1, the ligand forms a planar four-membered metallocycle. All three of the C–S distances in the ligand are identical within standard deviation. The structure, therefore, involves an unusual bridging coordination mode for a thioxanthate ligand.[16] Upon expanding the coordination sphere around Mo_2, all the Mo–S and Mo–C_{Cp} distances have increased slightly. For

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(9) Casewit, C. J.; Rakowski DuBois, M. *J. Am. Chem. Soc.* **1986**, *108*, 5482.
(10) DuBois, D. L.; Miller, W. K.; Rakowski DuBois, M. *J. Am. Chem. Soc.* **1981**, *103*, 3429 and references therein.
(11) After the reaction was stirred for 15 h, the precipitated product was filtered and washed with THF. Mass spectrum (EI), m/e^+ 548 ($P - CH_3I$), 478 ($MeCp_2Mo_2S_4$), 142 (CH_3I). Anal. Calcd for $C_{16}H_{19}Mo_2S_5I$: C, 27.84; H, 2.77; S, 23.22. Found: C, 27.89; H, 2.66; S, 23.19.
(12) Gunther, H. *NMR Spectroscopy*; translated by Gleason, R. W.; Wiley: New York, 1980; p 243.
(13) ^{13}C NMR ($CDCl_3$, 250 MHz, $T = -50$ °C) 16.6, 16.3 (Me groups); 92.1, 96.9, 101.8, 105.4 (Cp's); 147.3 ppm (SC_2H_2S); a very weak resonance at 174 ppm is tentatively assigned to C–Mo; ($T = 30$ °C) 16.5 (SMe); 147.4 (SC_2H_2S); 174 ppm (C–Mo). Resonances associated with the MeCp ligands are not observed at this temperature.
(14) A single crystal of $[(MeCpMo)_2(SCHCHS)(S_2CSMe)]I$, obtained by slow evaporation of a CH_2Cl_2 solution, had cell dimensions $a = 11.676$ (3) Å, $b = 13.992$ (4) Å, $c = 14.286$ (2) Å, and $\beta = 116.26$ (1)°; $V = 2093.0$ (8) Å^3, $Z = 4$, $\rho_{calcd} = 2.19$ g/cm^3. By use of a crystal of dimensions 0.3 × 0.3 × 0.2 mm, 8119 reflections were measured at values of $\pm h$, $+k$, and $\pm l$ in the range $3.0° \leq 2\theta < 50°$; 3122 of the 3707 unique reflections had $F_o \geq 6\sigma F_o$. The structure was solved by the heavy atom method and Fourier techniques. Full-matrix least-squares refinement on 229 variables with all non-hydrogen atoms anisotropic converged with residuals of $R = 0.046$ and $R_w = 0.061$. Atomic parameters and observed and calculated structure factors are included in the supplementary material.
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