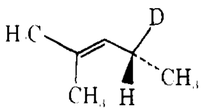
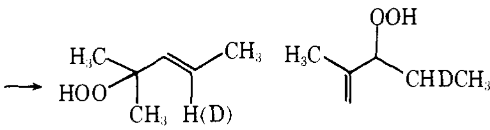
sitions as revealed in **5** by the isotope ratios for cis and trans competition.

Perhaps most intriguing is the finding of an isotope effect in **8** below. Again we have a case where D is presented to the



8



$k_H/k_D = 1.2$

top face of the olefin, H to the bottom face. Here, however, in contrast to the transformation **2** → **3** the adjacent *cis*-CH_3 group is equally as reactive as the chiral methylene unit. Thus the top face leads to an isotope effect of 1.4 (a cis competition) and the bottom face a normal product ratio since isotopes are not competing. With our earlier contention that top and bottom faces are equally reactive and noncompetitive, this neatly explains the ratio $k_H/k_D = 1.2$, which is an average of 1.4 (top) with 1.0 (bottom).

Were it not for the recent compelling calculations of Goddard and Harding,[10] which conclude that perepoxides are energetically inaccessible in the reaction, we could claim support here for this intermediate. Our results do, at a minimum, provide strong evidence for some intermediate in the reaction, an intermediate with structural requirements not dissimilar to those of the unknown perepoxides.

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Kinetics of Disulfide Cleavage by Methylmercury. Evidence for a Concomitant Electrophilic and Nucleophilic Mechanism

Sir:

The realization that methylmercury is produced in living systems from a variety of organic and inorganic mercury compounds has greatly increased the interest in its chemistry. Rapid ligand exchange reactions play a key role in the bioavailability of CH_3Hg^{II} derivatives which are bound almost exclusively to sulfhydryl ligands.[1,2] Mercaptide anion exchange in RHgSR′–RHgSR″ systems is also surprisingly rapid and provides yet another pathway for the migration of organomercurials in nature.[2] Since the disulfide linkage of cystine is probably the only covalent cross-linkage in most proteins and peptides, the reaction of the –SS– moiety with CH_3Hg^{II} could have a profound effect on the tertiary structure of a large variety of natural products.

The most commonly encountered electrophiles in –SS– bond cleavage are H^+ and metal ions with a high affinity for sulfur, such as Ag^+ and Hg^{2+}.[3] For example, silver ion has been used to good advantage by Davis[4a] in the synthesis of sulfenamides from disulfides and mercury catalysis has been employed in peptide synthesis.[4b] We found no reports of a systematic mechanistic study of metal catalysis of disulfide cleavage.[5] The dual objective of this study is to establish the role of CH_3Hg^{II} in reactions with the ubiquitous disulfide linkage and to provide kinetic evidence for a metal-assisted concomitant cleavage of this functional group.

There are two fundamental mechanistic pathways for heterolytic cleavage of the disulfide bond.[6,7] The first involves attack at sulfur by a nucleophile with displacement of RS^- (eq 1) and the second results from the combined catalysis of both an electrophile and a nucleophile (eq 2).

$$\mathrm{RSSR + Nu^- \longrightarrow RSNu + RS^-} \qquad (1)$$

$$\mathrm{RSSR + E^+ \rightleftharpoons RS\overset{+}{S}(E)R \xrightarrow{Nu^-} RSNu + RSE} \qquad (2)$$

In a definitive study involving base-catalyzed isotopic exchange between thiol and disulfide, Fava[10a] found that simple S_N2 displacement at sulfenyl sulfur exhibited a second-order rate expression of the form rate = $k_2[RSSR][RS^-]$. The isotopic exchange was also catalyzed by HX ($I^- > Br^- > Cl^-$) but not by the more acidic, but weakly nucleophilic, perchloric acid.[6a] Thorough kinetic studies by Kice on sulfur–sulfur bond scission have firmly established the existence of an acid-catalyzed concomitant –SS– bond rupture involving protonation of one sulfur and nucleophilic displacement at the other (eq 2).[6b,c,11]

There are relatively few analytical methods available that provide accurate rate measurements for disulfide cleavage.[6d] As reported[11a] in a recent kinetic study of the acid-catalyzed disproportionation of an unsymmetrical disulfide, the continuous monitoring of the relative intensity of NMR signals failed to give high quality kinetic data. We experienced similar difficulties but were able to circumvent the problem by monitoring the intensity of the recorder output signal of the NMR with an electronic integrator of the type that is typically used to measure peak areas in gas chromatograms.[12]

Our objectives were realized by examining the cleavage of dimethyl disulfide by the action of methylmercury acetate and triethyl phosphite.[13] The rate of –SS– bond scission was followed in the NMR by continuously measuring the loss of CH_3SSCH_3 (137 Hz) and the formation of CH_3HgSCH_3 (136 Hz) (eq 3). A second mole of CH_3Hg^+ is consumed in a second rapid reaction (eq 4).[14] Our results for –SS– bond breakage under both psuedo-first- and psuedo-second-order conditions are listed in Table I.

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Table I. Rate of Cleavage of Dimethyl Disulfide by Methylmercury Acetate in the Presence of Triethyl Phosphite[a]

expt	CH_3SSCH_3, M	CH_3HgOAc, M	$t_{1/2}$, min	k_1, s^{-1}	error,[b] %	corr coeff
1	0.050	0.500	45	2.55×10^{-4}	3.9	0.979
2	0.050	0.500	43	2.66×10^{-4}	4.1	0.991
3	0.050	0.500	41	2.85×10^{-4}	3.0	0.994
				k_{obsd}, $mol^{-1}\ s^{-1}$		
4	0.025	0.050	52	1.28×10^{-2}	4.0	0.984
5	0.038	0.076	38	1.16×10^{-2}	3.3	0.993
6	0.050	0.100	29	1.13×10^{-2}	3.0	0.987
7	0.050	0.100	25	1.33×10^{-2}	2.4	0.990
8	0.050	0.100	25	1.33×10^{-2}	2.6	0.991
9	0.050	0.100	26	1.26×10^{-2}	3.3	0.991
10	0.075	0.150	21	1.04×10^{-2}	3.6	0.992

[a] All data were collected in CH_2Cl_2 solvent where the $P(OEt)_3$ concentration was 1.0 M and the temperature of kinetic runs 1–3 and 4–10 was 15 and 41 °C, respectively. [b] The percent error is based upon the standard deviation of the least-squares plot.

$$CH_3SSCH_3 + CH_3HgOAc \overset{\text{fast}}{\rightleftharpoons} CH_3\overset{+}{S}(HgCH_3)SCH_3\ \bar{O}Ac$$

1

$$\xrightarrow[P(OEt)_3]{k_2,\ \text{slow}} CH_3HgSCH_3 + CH_3\overset{+}{S}P(OEt)_3\ \bar{O}Ac \quad (3)$$

2

$$CH_3\overset{+}{S}P(OEt)_3\ \bar{O}Ac + CH_3HgOAc$$

2

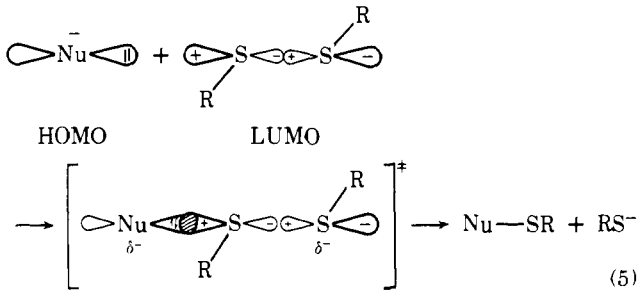
$$\xrightarrow{\text{fast}} CH_3HgSCH_3 + Ac_2O + O{=}P(OEt)_3 \quad (4)$$

The pseudo-first-order data, where the rate is $k_1[CH_3SSCH_3]$, was obtained while maintaining an excess of both CH_3Hg^+ and $P(OEt)_3$. The rate constant $k_1 = 2.7_1 (\pm 0.24) \times 10^{-4}\ s^{-1}$ was determined in methylene chloride at 15 °C by using the rate expression $\ln [(C_1 + C)/C_1] = k_1 t$, where C_1 is the integrated area for the methyl resonances of CH_3SSCH_3 and C represents the area for the methyl mercaptide protons in $CH_3SH_gCH_3$. All plots were linear and the correlation coefficients along with other data are given in Table I.

For the pseudo-second-order kinetics the rate is given by $k_{obsd}[CH_3SSCH_3][CH_3Hg^+]$ where $k_{obsd} = K_{eq}(2)k_2[P(OEt)_3]$ and K_{eq} is the equilibrium constant for the formation of sulfonium ion **1**. The rate constants were obtained from the expression $(C/C_1)(1/a) = k_{obsd}t$ where a is the initial concentration of CH_3SSCH_3. A plot of the half-lives calculated for the initial concentrations vs. $1/a$ afforded, as the reciprocal of the slope, the rate constant $k_{obsd} = 1.24 \times 10^{-2}\ mol^{-1}\ s^{-1}$.

Control experiments have established that neither CH_3HgOAc nor triethyl phosphite will effect disulfide bond cleavage under our conditions.[15] Similarly, ionic mercurials such as CH_3HgNO_3, in the absence of added nucleophile, or highly associated ones like CH_3HgI where the concentration of CH_3Hg^+ is minimal, fail to catalyze -SS- bond scission. We have also found that CH_3SSCH_3 is not competing with $P(OEt)_3$ as the nucleophile at temperatures up to 80 °C. We conclude from these experimental facts that the metal-assisted -SS- bond rupture is first order in both CH_3Hg^+ and disulfide. These data are consistent with a bimolecular rate limiting S_N2 attack by $P(OEt)_3$ on the sulfonium ion **1** (eq 3).

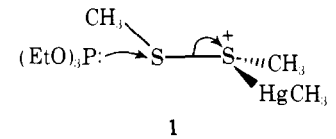
The suggested mechanism may also be rationalized on the basis of fundamental theoretical considerations. Frontier orbital theory suggests that nucleophilic -SS- bond cleavage (eq 1) proceeds by attack of the lone pair of the Nu^- (HOMO) on the empty σ^* orbital of the -SS- bond (LUMO).[16] Transfer of electron density from the nucleophile to the antibonding σ^* MO will effect -SS- bond rupture and displacement of RS^- (eq 5). The reaction is, in principle, comparable with a typical



(5)

S_N2 displacement at saturated carbon and, by analogy, there is no need to invoke a negatively charged intermediate or d-orbital participation for the -SS- bond breakage.[17] In a particularly interesting report by Kice,[18] it was suggested that nucleophilic displacement at sulfenyl sulfur is at least 10^9–10^{20} faster than displacement at sp^3 carbon, thereby reflecting the relatively low (~42 kcal/mol) sulfur-sulfur bond energy.[19]

Ab initio calculations[20] have provided convincing evidence that the role of the proton in concomitant -SS- scission (eq 2) is to effect an increase in the electron affinity of the disulfide, as seen by a marked decrease in the energy of LUMO, and to transform RSE (eq 2) into a neutral leaving group. We have found that CH_3Hg^+ is highly effective in initiating cleavage of the -SS- bond and we attribute this to a similar lowering of the energy of the -SS- σ^* orbital upon formation of the sulfonium ion **1**. This reduction in energy difference between the frontier molecular orbitals involved in displacement will facilitate the approach of $P(OEt)_3$ (HOMO) and increase the rate of displacement on the σ^* orbital of **1** (eq 6).



1

HOMO (n) LUMO (σ^*)

$$\longrightarrow (EtO)_3\overset{+}{P}SCH_3 + CH_3HgSCH_3 \quad (6)$$

In conclusion, we have delineated some of the reaction requirements for -SS- bond cleavage by CH_3Hg^{II} in a simple disulfide and developed a model system for the study of the interaction of this important functional group with CH_3Hg^{II}. Our kinetic study provides the first mechanistic evidence for a concomitant metal-assisted disulfide cleavage.

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(13) The use of OH^- as the nucleophile is complicated by the secondary reactions of RSOH and secondary amines gave nonlinear kinetic plots. Triethyl phosphite appears to be a more effective nucleophile for –SS– cleavage than the oxygen or nitrogen nucleophiles. We have previously characterized CH_3HgSCH_3.[2] We have isolated acetic anhydride and triethyl phosphite, in addition to $CH_3HgSC_6H_5$ (75%), from the reaction mixture of the cleavage of diphenyl disulfide. The NMR chemical shifts are relative to Me_4Si.
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Structure of Preuroporphyrinogen. Exploration of an Enzyme Mechanism by ^{13}C and ^{15}N NMR Spectroscopy

Sir:

We recently described[1,2] the discovery of preuroporphyrinogen (preuro'gen), a labile ($t_{1/2}^{37°C}$ = 4 min), tetrapyrrolic intermediate in the conversion of porphobilinogen (PBG, **1**) to uroporphyrinogen (uro'gen) I (**2**) catalyzed by the enzyme PBG deaminase. The importance of this substance, released from the enzyme and observed as a ^{13}C-enriched species at pH 8.5, resides in its proven role[2] as the first recognized substrate for the second enzyme of tetrapyrrole biosynthesis, uro'gen III cosynthetase, which has hitherto been considered as a synergistic companion for deaminase, since both enzymes[3] are required to convert PBG to uro'gen III (**3**), the precursor of heme,[4] sirohydrochlorin,[5] chlorophylls,[4] and vitamin B_{12}.[4-6]

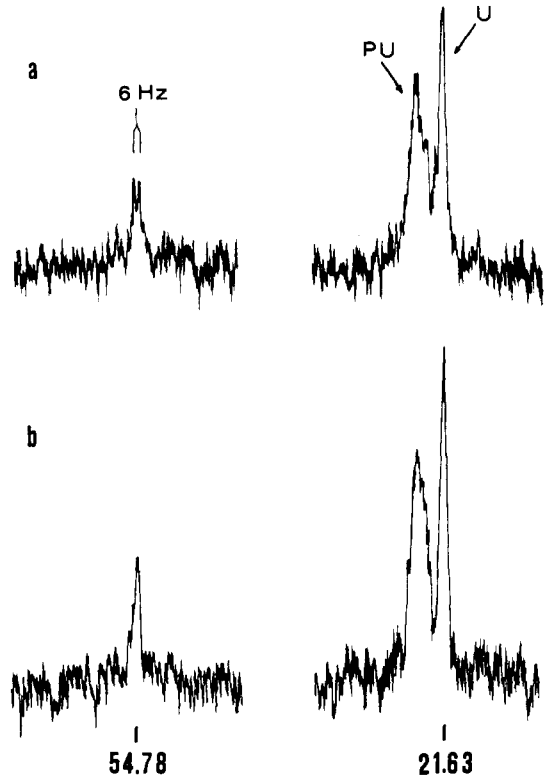

Figure 1. Proton decoupled 75.5-MHz ^{13}C spectra at 0 °C of 3-min incubations (37 °C, 85% conversion) of PBG deaminase with [11-^{13}C:1-^{15}N]-PBG (a) and [11-^{13}C]-PBG (b). Spectrum a is the result of 6000 and b of 2200 90° pulses accumulated over a spectral width of 7500 Hz while locked to internal D_2O (10%) with a repetition rate of 0.8 s. The lines were broadened 2 Hz by exponential multiplication of the FID.

By observing the ^{13}C NMR spectra of incubations of [11-^{13}C]- and of [2,11-$^{13}C_2$]-PBG with deaminase it was possible to detect, in addition to the signals assigned to uro'gen I (**2**), ^{13}C enrichments for four and eight carbons, respectively, of preuro'gen. Three structures, **4**, **5** (X = OH), and **6**, compatible with these chemical shifts and lack of ^{13}C–^{13}C coupling were proposed,[1] the N-alkylated macrocycle (**4**) being preferred for reasons stated elsewhere.[1,2] The structure **4** for preuro'gen has now been proved by using [11-^{13}C:1-^{15}N]-PBG as the substrate for deaminase and observing both ^{13}C and ^{15}N NMR spectra at the point of maximum preuro'gen formation.

The doubly enriched PBG was synthesized by modification of literature methods[7,8] and contained 90% ^{13}C at C-11 and 99% ^{15}N at N-1. Incubation[9] of 0.4 mg of this substrate with highly purified deaminase from *Rhodopseudomonas spheroides* (450 units/ml) for three minutes (37 °C; 85% conversion) gave the ^{13}C NMR spectrum shown in Figure 1a. In addition to the methylene signals (U) for uro'gen I at δ 21.63 ppm,[10] for C-11 of the remaining (15%) PBG at δ 34.95 ppm and for three carbons of preuro'gen (PU) at δ 22.00 ppm, the spectrum shows a doublet centered at δ 54.78 ppm (J = 6 Hz) which by comparison with the singlet observed for this resonance (see Figure 1b) in the non-^{15}N labeled experiment, must be ascribed to one bond ^{13}C–^{15}N coupling[11] with one of the enriched (^{15}N-1) pyrrolic nitrogens. Upon heating to 37 °C the latter signal disappears along with the three carbon methylene enrichment at δ 22.00 ppm, as preuro'gen is converted to uro'gen I. Confirmation of the above interpretation was secured by repeating the experiment whilst observing the ^{15}N NMR spectrum (at 8.1 MHz) which is shown in Figure 2. In addition to singlets for the four pyrrolic nitrogens of ur-

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