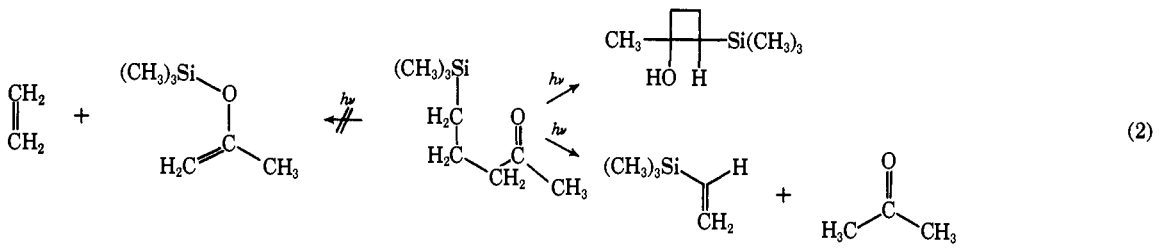
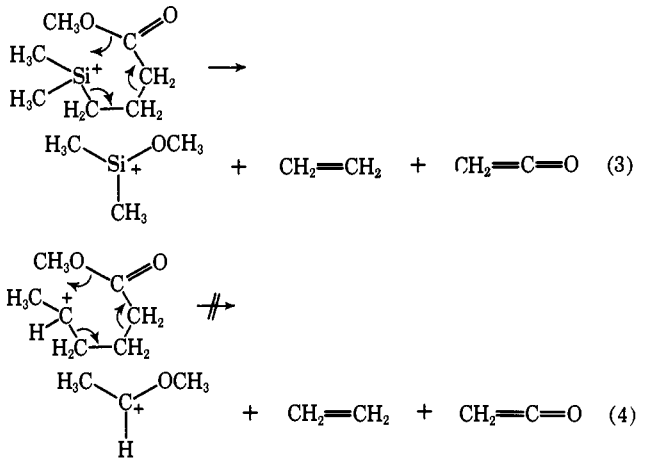


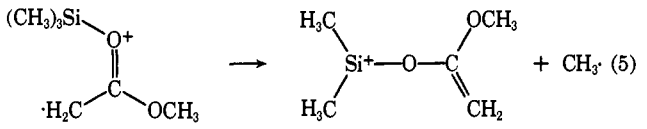
(2)



(3)

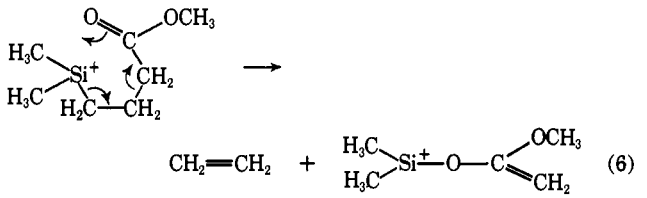
(4)

146 = 117.54) provides additional evidence in support of this process (eq 5).



(5)

An alternative fragmentation process which could have led to this same ion—previously favored by us[5]—involves attack of the carbonyl oxygen on the siliconium ion center of the ion *m/e* 159 with simultaneous loss of ethylene[17] (eq 6). While this rearrangement was attractive, it is *not* supported by the observation of a metastable ion at *m/e* 107.9 (calcd $131^2/159 = 107.9$). Hence, we must conclude that the ion *m/e* 131 arises by loss of a methyl radical from the silyl McLafferty rearrangement ion.



(6)

To verify the structures of these rearrangement ions, the mass spectra of the methyl-d_3 ester, as well as that of the methyl 4-trimethylsilyl-2,2-dideuteriobutyrate, were examined. They were completely in accord with the assigned structures.[18]

A possible driving force for the rearrangement of a methoxy group to the siliconium ion center is the high silicon–oxygen bond strength.[19] The greater strength of a silicon–oxygen bond compared to that of a hydrogen–oxygen or a carbon–oxygen bond must be important also in the silyl McLafferty rearrangement. The possibility that silicon can form a pentacoordinate transition state by use of its 3d orbitals may also favor this migration of the trimethylsilyl group.

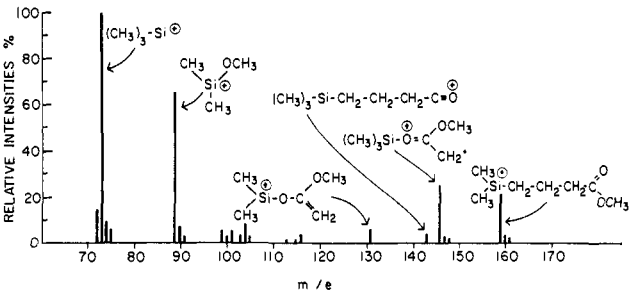


Figure 1. Methyl 4-trimethylsilylbutyrate.

Acknowledgments. We wish to acknowledge a National Science Foundation grant for purchase of the mass spectrometers. This work was also supported in part by the Research Corporation, and by Biomedical Support Grant No. FR-07012-02 from the National Institutes of Health. Finally, we thank Professor K. L. Servis for helpful discussions.

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Enzymatic and Nonenzymatic Demethylation of Methylcobalamin and of Abiogenic Cobaloxime Model Substrates. Methane Biosynthesis by *Methanobacillus omelianskii*[1]

Sir:

Extracts of the methanogenic bacterium *Methanobacillus omelianskii* (MOH)[2] have recently been shown[3] to utilize methylcobalamin and, most surprisingly, the completely abiogenic methyl cobaloximes as substrates for methane evolution. Reaction 1 shows an absolute requirement for catalytic amounts of ATP and factor III ((Co) denotes the cobaloxime, [Co] the cobinamide moiety).[4]

(17) This observation places serious doubt on the rearrangement process we have previously discussed for the formation of the *m/e* 105 ion in the mass spectrum of methyl 3-trimethylsilylproprionate.[5] Studies to clarify the source of this ion are continuing.

(18) A similar silyl–McLafferty rearrangement has been observed in the mass spectrum of 4-trimethylsilylbutyronitrile.

(19) C. Eaborn, "Organosilicon Compounds," Butterworth & Co., Ltd., London, 1960, p 90: Si–O, 108 kcal/mol; C–O, 85.5 kcal/mol.

(1) This research was supported by Grant GP 12324 of the National Science Foundation.

(2) M. P. Bryant, B. C. McBride, and R. S. Wolfe, *J. Bacteriol.*, **95**, 1118 (1968).

(3) B. C. McBride, J. M. Wood, J. W. Sibert, and G. N. Schrauzer, *J. Amer. Chem. Soc.*, **90**, 5276 (1968).

(4) Factor III is cobalt(III) 5-hydroxybenzimidazolylcobamide: J. M. Wood and R. S. Wolfe, *Biochemistry*, **5**, 3598 (1966), the natural cofactor. However, vitamin B_{12a}, the corresponding cobamide with

Table I. Relative Rates of Enzymatic (Column I) and Nonenzymatic (Column II) Methane Formation from Various Co-Methyl Substrates[a] and of the Nucleophilic Displacement of CH_3 Groups by CH_3S^- (Column III)

No.	In-plane ligand[b]	Axial base	Rel rate of CH_4 evolution: Enzymatic I	Rel rate of CH_4 evolution: Non-enzymatic II[c]	Rel rate[c] of nonenzymatic nucleophilic CH_3 group displacement by CH_3S^- III
1	(Methylcobalamin)		1.0	1.0	1.0
2	Dmg	H_2O	0.69	1.38	
3	Dpg	H_2O	0.63	1.20	
4	Dpg	Pyridine	0.625	2.60	
5	Dmg	Pyridine	0.552	0.60	4.0×10^{-4}
6	Dmg	Benzimidazole	0.230	0.17	
7	Dmg	$P(C_6H_5)_3$	0.222	0.17	9.06×10^{-3}
8	Dmg	CNC_6H_{11}	0.0855	0.075	
9	Glyoxime	Pyridine	0.079	0.73	
10	Cyclohexanedione dioxime	Pyridine	0.20	0.38	
11	Dmg BF_2	Pyridine	0	0.15	3.96×10^{-1}
12	Schiff base I	H_2O	0	0.63	
13	Schiff base II	H_2O	0	0.97	4.47×10^{-2}

[a] Column I: with cell extracts of *M. omelianskii*, most data from ref 2. Column II: relative rates of methane evolution at pH 4.6 with dithioerythritol as the reducing agent. Column III: Relative rates for reaction of Co–CH_3 compounds with CH_3SH (0.5 *M* in 1 *M* NaOH in CH_3OH). [b] Dmg = dimethylglyoxime; Dpg = diphenylglyoxime; Dmg BF_2 = BF_2 derivative of Dmg; Schiff base I = bis(biacetyl monoxime)propylenediimine(I,3); cobalt complex supplied as the perchlorate. Schiff base II = bis(salicylaldehyde)ethylenediimine. [c] Rates determined under pseudo-first-order conditions (large excess of thiol or mercaptide ion, respectively).

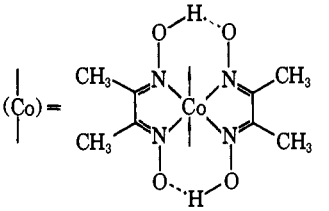
$$\begin{matrix}CH_3\\|\\(Co)\\|\\B\end{matrix} \xrightarrow[\text{(factor III enzyme)}]{\text{ATP, } H_2\text{, [Co]}} CH_4 + (Co^{II}) \qquad (1)$$

The efficiency of the substrates depends on the nature of the in-plane and axial ligands, reaching 69% of the specific activity of methylcobalamin in the case of methylaquobis(dimethylglyoxime)cobalt. The remarkable ability of the simple model compounds to function as substrates in the enzymatic reaction[3] poses two important mechanistic alternatives. The enzyme could either demethylate the substrate and transport the methyl group to the cobalt atom of factor III (mechanism I) or reductively demethylate the substrates at an active site of the enzyme as in the case of methylcobalamin (mechanism II). Initial experiments with labeled methylcobaloxime gave some indication of methylcobalamin formation if ATP was omitted but did not prove that this was a main reaction path.[3] In the following we report new experimental evidence demonstrating that mechanism II accounts for the formation of methane in this system.

The transfer of the methyl group from a cobaloxime to a cobinamide cobalt atom leads to mechanistic difficulties in view of the stability of the methylcobaloximes[5] and cannot take place directly. The only successful methyl group transfer has been verified in low yield by irradiation of methylcobaloxime in the presence of vitamin B_{12r}.[6] This methyl radical transfer reaction requires the presence of light, is inefficient, and thus could not account for the behavior of methylcobaloximes. A plausible indirect transfer mechanism consists in the removal of the methyl group by an intermediate carrier. This carrier could be an enzyme mercaptide group (*e.g.*, of homocysteine), removing the cobaloxime methyl group by nucleophilic attack and transporting it to reduced factor III enzyme *via* a methylsulfonium ion intermediate (*e.g.*, S-adenosyl,[6-8] eq 2).

$$\begin{matrix}CH_3\\|\\(Co)\\|\\B\end{matrix} + {}^-S{-}R \longrightarrow CH_3{-}S{-}R \xrightarrow[-P_i]{ATP}$$

$$CH_3{-}\overset{+}{S}(R)(A) \xrightarrow{[Co^I]\text{Enzyme}} \begin{matrix}CH_3\\|\\[Co]\text{enzyme}\end{matrix}$$

$$\begin{matrix}\\|\\(Co)=\end{matrix}$$  (2)

To establish if a nucleophilic displacement of the methyl group occurs in the enzymatic reaction, the relative rates of the reaction of methylcobaloximes with methylmercaptide ion were determined at room temperature in 1 *M* NaOH–CH_3OH. The relative rates vary over three orders of magnitude but show no relation to the specific activities in the methane production (Table I), in serious contradiction with mechanism I.

$$\begin{matrix}CH_3\\|\\(Co)\\|\\B\end{matrix} + \begin{matrix}HS\\HS\end{matrix} \xrightarrow{-B,\ -H^+}$$

$$\begin{matrix}CH_3\\|\\(Co)\\|\\{}^-S\\H{-}S\end{matrix} \xrightarrow{-(Co^I)^-,\ -H^+,\ S} CH_3^- \xrightarrow{+H^+} CH_4 \qquad (3)$$

5,6-dimethylbenzimidazole attached to cobalt, is also an active cofactor. Recent work (A. M. Roberton and R. S. Wolfe, *Biochim. Biophys. Acta*, **192**, 420 (1969)) reveals that only catalytic amounts of ATP are required and that stoichiometric ATP is not obligatory for methane synthesis.

(5) G. N. Schrauzer, *Accounts Chem. Res.*, **1**, 97 (1968).

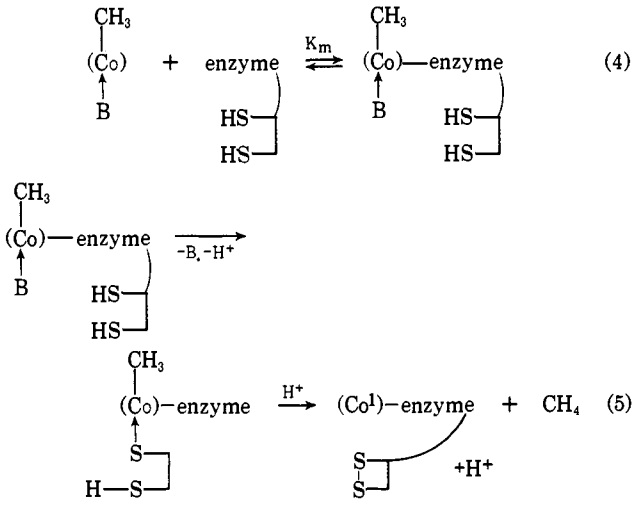
(6) G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Amer. Chem. Soc.*, **90**, 6681 (1968).

(7) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **89**, 3607 (1967).

(8) See F. M. Huennekens in "Biological Oxidations," T. P. Singer, Ed., Interscience Publishers, New York, N. Y., 1968, pp 482–513, for general discussion.

The ability of thiols such as dithioerythritol (DTE) to reductively cleave the Co–C bond in methylcobalamin and alkylcobaloximes (eq 3)[9] nonenzymatically provides a simple means of testing the validity of mechanism II.

The relative rates of methane evolution in aqueous solution at pH 4.6 with DTE as the sole reducing agent present are given in Table I, column II, revealing a definite correlation with the specific activities of the cobaloximes in the enzymatic process. This convincingly supports mechanism II and suggests that a thioprotein SH group provides the active site for the reductive demethylation of both methylcobalamin and methylcobaloximes in this enzyme system. Figure 1 shows that the substrates fall into two groups. Group A substrates are reactive enzymatically as well as nonenzymatically. Group B substrates are only active nonenzymatically. Group A substrates are all derived from unmodified bis(dimethylglyoximato)- or bis(diphenylglyoximato)cobalt complexes. The specific enzymatic activity appears to converge to a maximum rate equal to two-thirds of that of methylcobalamin, suggesting a smaller value of K_m for the cobaloxime model compounds. The relative rates of methane evolution increase in the order of axial bases CNC_6H_{11} < $P(C_6H_5)_3$ < benzimidazole < pyridine $\simeq$ H_2O. This is a sequence of decreasing stability of the axial base adducts,[10] suggesting that the axial base is displaced on interaction with the enzyme. Group B substrates are derived from modified bis(dimethylglyoximato) ligands (*e.g.*, by substituting the oxime protons with BF_2 groups) or from unrelated ligands (*e.g.*, of cobalt bis(salicylaldehyde)ethylenediimine). They are in most cases inactive presumably because of their weak binding to the active site of the enzyme. The principal steps in the enzymatic demethylation of methylcobalamin or of methylcobaloximes thus are described by eq 4 and 5.



(4)

(5)

The available evidence suggests that cobaloxime model compounds compete with corrins for the same enzyme binding site. The demethylation leaves the enzyme in the oxidized state; its conversion to the original reduced form requires the reduction of a disulfide group. Reduced corrins are much more efficient catalysts of thiol–disulfide oxidation–reduction than cobaloximes[11] and may be the catalysts in the reactivation of the active site. The methylcobaloximes are only demethylated and thus cannot completely replace the corrin cofactor. Methyl(aquo)rhodoxime, the rhodium analog of methyl(aquo)cobaloxime,[12] is a weak inhibitor of the enzymatic methane production with methylcobalamin as the substrate. Its nonenzymatic reductive demethylation with DTE proceeds at only one-fifth of the rate of methyl(aquo)cobaloxime under identical conditions. The different behavior of the rhodoxime is primarily a consequence of the greater thermodynamic stability of the Rh–C bond.

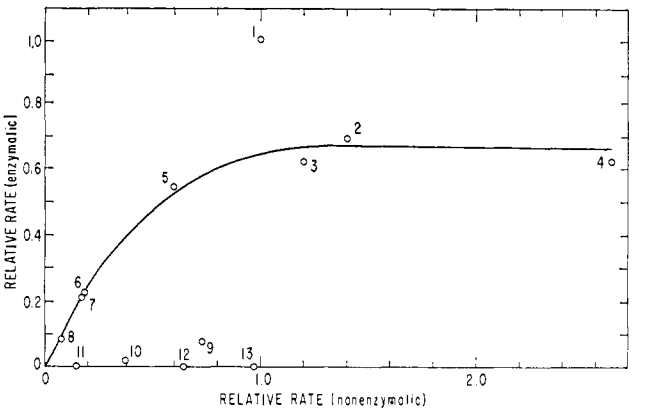


Figure 1. Relative rates of the nonenzymatic methane evolution *vs.* relative rates of the enzymatic methane evolution by cell extracts of *M. omelianskii* in the presence of ATP (10 μmol): TES buffer (100 μmol, pH 7.0), gas phase H_2, incubation temperature 40°. The numbers refer to the complexes listed in Table I.

The present work has led to the postulate of a mechanism of reductive Co–C bond cleavage in a methane-producing enzyme which may be the prototype of other thioredoxine-B_{12} dependent reductases (*i.e.*, of ribonucleosides). It also strikingly demonstrates the usefulness of cobaloximes in the study of vitamin B_{12} dependent enzymatic processes.

Acknowledgment. We thank Drs. J. M. Wood and B. C. McBride (Urbana) for determining the enzymatic activity of the cobaloxime substrates.

(9) G. N. Schrauzer and J. W. Sibert, submitted for publication. For initial work on the reductive cleavage of Co–C bonds by thiols, see G. N. Schrauzer and R. J. Windgassen, *J. Amer. Chem. Soc.*, **89**, 1999, 3607 (1967).

(10) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **88**, 3738 (1966).

(11) G. N. Schrauzer and J. W. Sibert, *Arch. Biochem. Biophys.*, **130**, 257 (1969).

(12) J. H. Weber and G. N. Schrauzer, *J. Amer. Chem. Soc.*, **92**, 726 (1970).

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Conformation of Metalloporphyrins in Solution

Sir:

In recent years there have been a number of X-ray crystallographic studies carried out on metalloporphyrins. The most interesting result from the studies completed to date is the variation of planar or nonplanar conformation of the metalloporphyrin depending on the metal involved and the nature of the extra planar ligand or ligands involved. We wish to report here a method, based on the high-field shift of the coordinated extra planar ligand in the proton magnetic resonance