111. Coenzyme F430 from Methanogenic Bacteria: Mechanistic Studies on the Reductive Cleavage of Sulfonium Ions Catalyzed by F430 Pentamethyl Ester

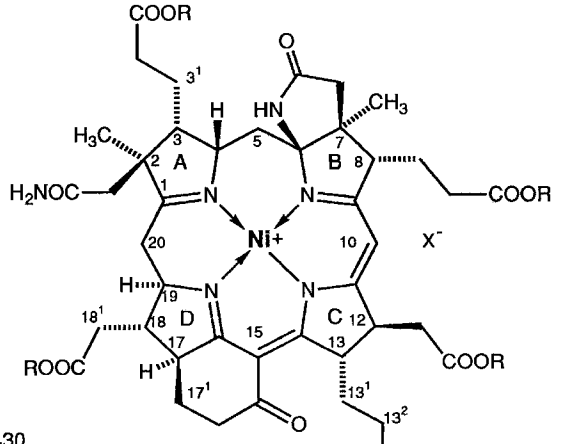
by **Shu-Kun Lin**[1]) and **Bernhard Jaun***

Laboratorium für organische Chemie, Eidgenössische Technische Hochschule, Universitätstrasse 16, CH–8092 Zürich

(14.V.92)

Mechanistic questions regarding the reductive cleavage of sulfonium ions by the Ni^{I} form of coenzyme F430 pentamethyl ester (F430M) were addressed in a series of kinetic studies and isotope labeling experiments. In neat DMF, methane formation from dialkyl(methyl)sulfonium ions consistently showed a delay time of *ca.* 1 h. In the presence of excess propanethiol, no delay was observed and methane formation followed pseudo-first-order kinetics with a logarithmic dependence of the initial rate on the concentration of propanethiol. From the temperature dependence of the reaction rate, an estimate for the activation parameters of $\Delta H^{\neq} = 49$ kJ mol^{-1} and (apparent) $\Delta S^{\neq} = -114$ J K^{-1} mol^{-1} was derived. The observation of deuterium incorporation into methane from $(CH_3)_2CHOD$, but not from $(CH_3)_2CDOH$, indicates that the fourth H-entity is introduced into CH_4 as a proton, and that free CH_3 radicals are not involved. In contrast to the reaction with the homogeneous one-electron reductant sodium naphthalide, the F430M-catalyzed reduction of mixed dialkyl(methyl)sulfonium ions showed a pronounced selectivity for the cleavage of Me–S over that of alkyl-S (alkyl ≠ Me) bonds. Mechanisms that are consistent with these results, as well as possible explanations for the time delay and the apparent highly negative entropy of activation, are discussed.

1. Introduction. – The hydrocorphinoid nickel complex **1** (F430) [1] is the cofactor of methyl-coenzyme M reductase which catalyzes the last step of methane formation in methanogenic bacteria according to *Scheme 1a* [2]. The mechanism by which the enzyme

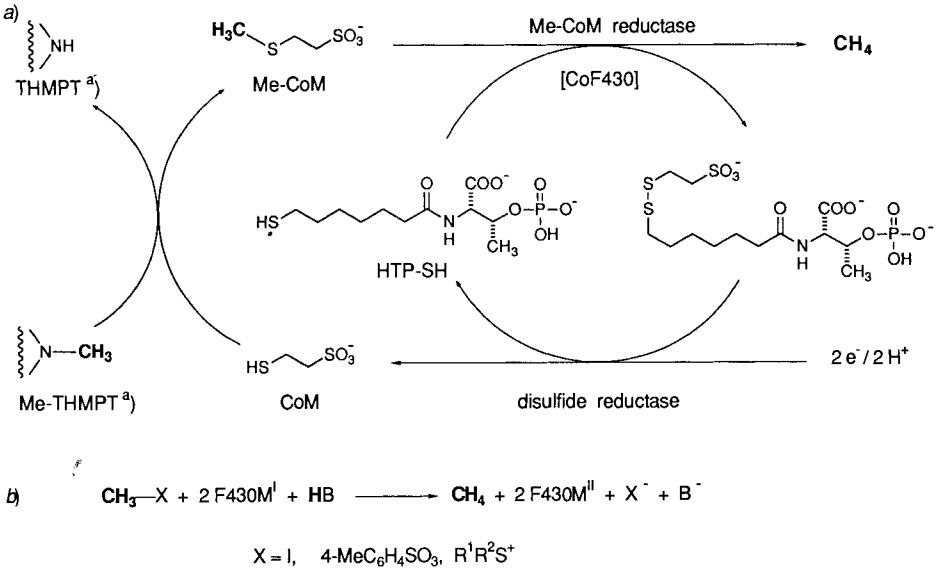


1 R = H; coenzyme F430

2 R = CH_3, X = ClO_4; F430M (= $F430M^{II}$)

[1]) Part of the planned Dissertation of *Sh.-K. L.*, ETH-Zürich, 1992.

Scheme 1



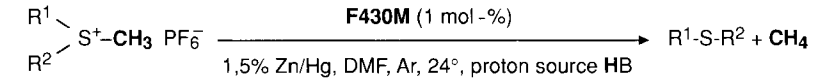
a) Me-THMPT = methyl-tetrahydro-methanopterin [2c].

catalyzes this reaction is still unknown. However, recent results by *Rospert et al.* [3] indicate that the active enzyme contains coenzyme F430 in its Ni^I form, which could be identified, because its EPR signal in the enzyme (MCR_{red1}) closely matched the spectrum of $F430M^I$ [4].

In a previous communication [5], we reported that the Ni^I form ($F430M^I$) of F430 pentamethyl ester ($F430M^{II}$; **2**) reacts with electrophilic methyl donors like MeI, MeOTs, and methylsulfonium ions to give methane according to *Scheme 1b*. Since methyl thioethers, including methyl-coenzyme M, do not react with $F430M^I$, the catalytic reduction of sulfonium ions in the presence of F430M (**2**) is still the only known case of a C–S bond being cleaved by a derivative of F430. This prompted us to carry out a more detailed study on the nature of this reaction, the results of which form the subject of this communication.

2. Kinetics of Methane Formation. – The reduction potential of zinc amalgam (Zn/Hg) is sufficiently cathodic to permit complete reduction of F430M (**2**) to the Ni^I form. But, in contrast to Na/Hg, Zn/Hg does not cause overreduction under attack of the ligand chromophore. Control reactions showed that, in the absence of **2**, only trace amounts of methane were produced, if sulfonium ions were stirred with Zn/Hg in DMF for several days. Therefore, the reductive cleavage of sulfonium ions R_2MeS^+ by $F430M^I$ could be studied using a catalytic system containing the sulfonium ion, Zn/Hg, and 1–2 mol-% of F430M according to *Scheme 2* [5]. Under conditions of vigorous stirring, reduction of **2** was fast enough to reach a steady state of nearly 100% $F430M^I$ (UV/VIS) in this reaction system.

Scheme 2



We reported [5] that, in neat DMF, the formation of methane from *S*-methylthiolanium hexafluorophosphate ($R^1,R^2 = -(CH_2)_4-$) showed a substantial lag of *ca.* 1 h, which was not observed in the presence of either a thiol, hydroxide, or Et_3N. This delay time was found to be a general phenomenon with all sulfonium ions studied. *Fig. 1a* illustrates the dependence of the kinetics of methane formation from Me_3S^+ on the concentration of propanethiol (PrSH). If the initial rate was plotted against the concentration of PrSH, a saturation curve with a logarithmic dependence was obtained (*Fig. 1b*) which would be consistent with a pre-equilibrium involving PrSH. In the presence of a large excess[2]) of PrSH, methane formation followed pseudo-first-order kinetics from the start of the reaction. The rate was found to depend linearly on the concentration of both, F430M and the sulfonium ion.

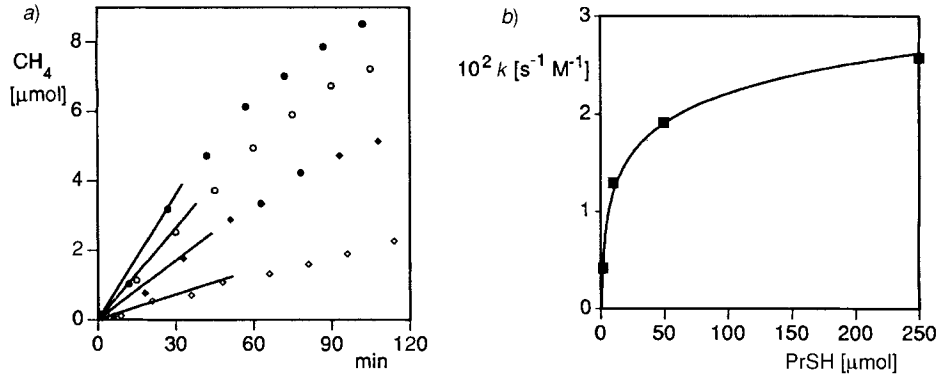


Fig. 1. a) *Dependence of the kinetics of methane formation from* Me_3S^+ *on the concentration of PrSH* (PrSH: 2 μmol (◇), 10 μmol (◆), 50 μmol (○), and 250 μmol (●)) *and* b) *initial rate* vs. *the concentration of PrSH*

The temperature dependence of the methane-formation rate was determined for three points within the limited accessible range of 0 to 50°[3]) (*Table 1, Fig. 2*). Assuming a second-order rate law according to *Eqn. 1*, an estimate of the activation parameters with $\Delta H^{\neq} = 49$ kJ mol^{-1} and $\Delta S^{\neq} = -114$ J K^{-1} mol^{-1} was obtained. Whereas the activation enthalpy was close to the value of 50 kJ mol^{-1} determined by *Bakac* and *Espenson* [6] for the reaction of $[Ni^I(tmc)]^+$ (tmc = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane) with 6-bromohex-1-ene, the (apparent) entropy of activation was much larger than typical values for comparable second-order reactions (see *Discussion*).

$$d[CH_4]/dt = k\,[F430M^I]\,[Me_3S^+] \quad (1)$$

[2]) Larger than *ca.* 5 times the amount of F430M (**2**).

[3]) Below 0°, the steady-state assumption regarding $F430M^I$ broke down, because the amalgam was near the freezing point, and reduction of **2** was slow. Above 50°, **2** was degrading fast enough to affect the kinetic results.

Table 1. *Kinetics of Methane Formation by Reductive Cleavage of Trimethylsulfonium Ion with F430M*[1a])

T [K]	325.2	296.2	273.2
$10^5 \cdot k'$ [s^{-1}][b])	4.832	0.8346	0.1508
$10^2 \cdot k$ [$M^{-1}s^{-1}$]	10.64	1.838	0.3322

[a]) Conditions as in *Fig. 2*. [b]) Pseudo-first-order rate constant.

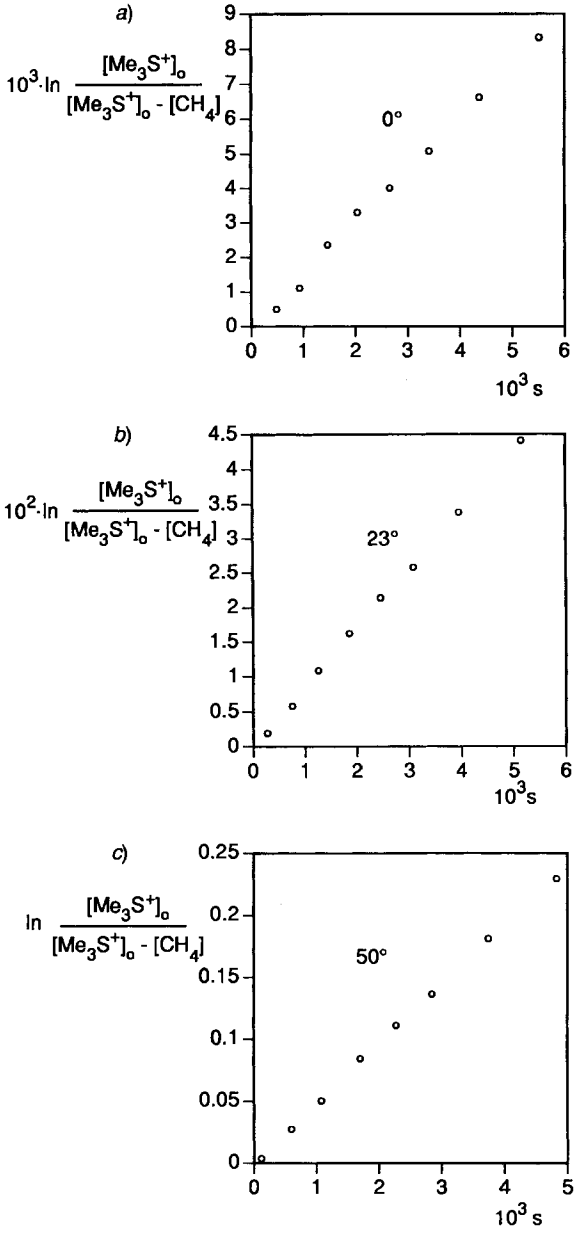
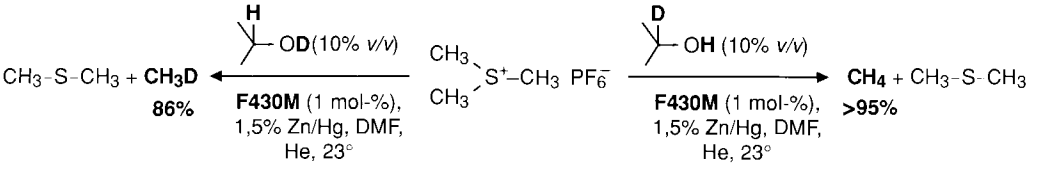


Fig. 2. *Temperature dependence of the rate of methane formation.* Conditions: F430M[I] (0.454 μmol, $4.54 \cdot 10^{-4}$ M) and $(Me_3S)PF_6$ (100 μmol, 0.1M) in 1 ml of DMF, 160 μl Zn/Hg; *a*) 0°, *b*) 23°, and *c*) 50°.

3. Deuterium-Incorporation Studies. – The question, whether the fourth H-entity of methane is introduced as an atom or as a cation was addressed by a series of deuterium-incorporation studies. In the presence of an excess of deuterated weak acids like RSD and R_3ND^+, the resulting methane was monodeuterated to 85–95% as determined by GC/MS, whereas no D was incorporated if the reaction was run in (D_7)DMF [5].

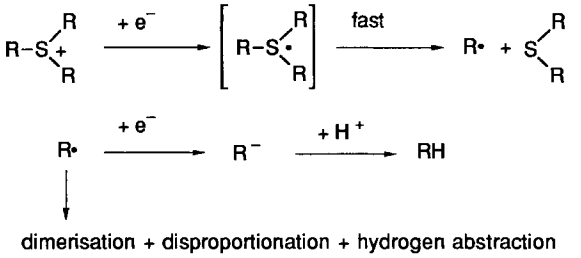
In two parallel runs, carried out in the presence of 10% (*v/v*) of two isotopomers of propan-2-ol as shown in *Scheme 3*, no D was incorporated from $(CH_3)_2CDOH$, whereas with $(CH_3)_2CHOD$ more than 85% of the resulting methane was CH_3D. Since the homolytical bond dissociation energy for H–C(2) of propan-2-ol (*ca.* 90 kcal mol^{-1}) is much smaller than that for the O–H bond (*ca.* 105 kcal mol^{-1}) [7], C-centered radicals react with propan-2-ol by abstraction of H–C(2) [8], while strong bases deprotonate the OH group. Therefore, the experiment summarized in *Scheme 3* demonstrates that the final step of the F430M-catalyzed cleavage of sulfonium ions into methane and a thioether must be a protonation and that a pathway *via* free Me radicals and H-abstraction can be ruled out.

Scheme 3



4. Selectivity for Methyl–Sulfur Bond Cleavage in Mixed Sulfonium Ions. – For the electrolytical reduction of sulfonium ions, which occurs only at potentials distinctly more cathodic than $E°(F430M^{II}/F430M^{I})$ [4], a mechanism according to *Scheme 4* was proposed [9]. The observation of *Wataki et al.* [10] that electrolysis of the sulfonium ion *S*-methylmethionine preferentially led to the product resulting from cleavage of the

Scheme 4

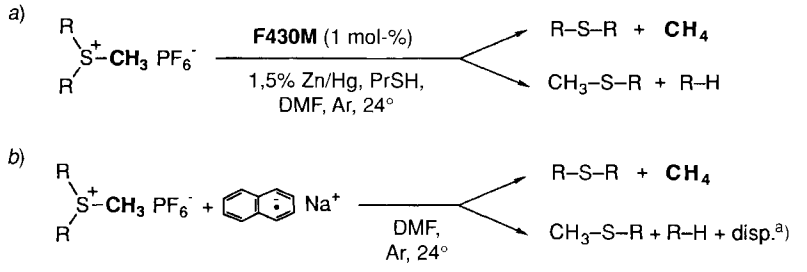


CH_2–S bond is consistent with the mechanism of *Scheme 4*, from which a product distribution determined by the stability of the generated alkyl radicals would be expected.

According to the deuterium-incorporation studies, the F430M-catalyzed reductive cleavage of sulfonium ions (*Scheme 5a*) follows a different mechanism. In fact, the

product distributions obtained in the presence of F430M with mixed sulfonium ions (*Table 2*) show a pronounced preference for the cleavage of the Me–S over that of the CH_2–S or CH–S bonds. As a reference reaction, the reduction by the strong one-electron reducing agent sodium naphthalide in dimethoxyethane (DME) was investigated (*Scheme 5b*). In contrast to the F430M-catalyzed reaction, the (presumed) outer-sphere electron transfer led to a product distribution (*Table 2*) that is consistent with either statistics (Me *vs.* Et or Pr) or radical stability (Me *vs.* i-Pr) as the product-determining factors.

Scheme 5



a) Products of radical disproportionation.

Table 2. *Product Distribution in the Reductive Cleavage of R_2MeS^+ Using the F430M-Catalyzed Reaction (Scheme 5a) and Reduction by Sodium Naphthalide (Scheme 5b)*

R	F430M-Catalyzed reduction		Reduction by $Na(C_{10}H_8)$	
	CH_4 [%]	R-H [%]	CH_4 [%]	R-H + disp[a]) [%]
Et	88	12	28	72
Pr	98.7	1.3	40	60
i-Pr	99.3	0.7	9	91

[a]) Products of radical disproportionation.

5. Isotope Effects. – In the F430M-catalyzed reduction of $(Me_3S)PF_6$, the overall methane-formation rate showed a significant isotope effect if i-PrOD was the deuteron source[4]). With PrSD, however, no isotope effect on the methane-formation rate was detectable (*Fig. 3, Table 3*). In competitive experiments, where either i-PrOD/i-PrOH 1:1 or PrSD/PrSH 1:1 were present, the product distribution CH_3D/CH_4 showed strong isotope effects for both proton donors (*Table 3*). The observation of different isotope effects on the overall reaction rate on one hand and on the product distribution on the other hand necessarily means that an intermediate, which is transformed into methane by the final protonation step, must be formed.

---

[4]) In the presence of propan-2-ol, methane formation showed a somewhat reduced time lag, as compared with the reaction in neat DMF. The isotope effect on the overall reaction rate was determined by comparison of the rates after the lag, where a pseudo-first-order rate law was obeyed.

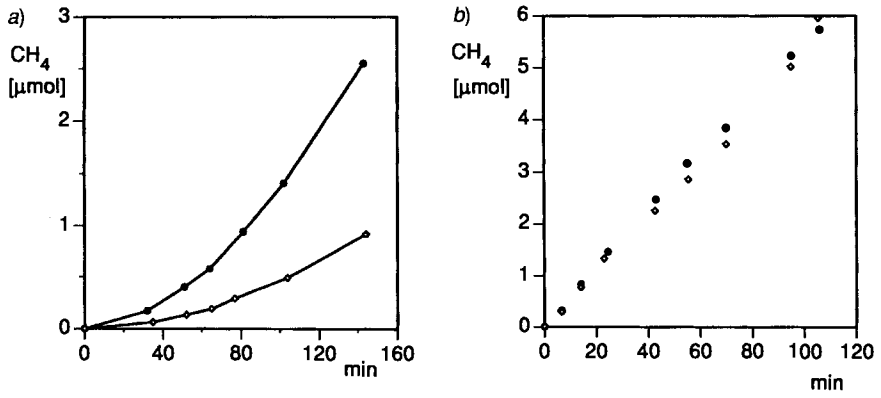


Fig. 3. *Kinetic isotope effect on the rate of methane formation observed in the presence of* a) *i-PrOD* (●) vs. *i-PrOD* (◇) *and* b) *PrSH* (●) vs. *PrSD* (◇). Conditions: see *Exper. Part.*

Table 3. *Isotope Effects on the Methane-Formation Rate and on the Product Distribution in the F430M-Catalyzed Reductive Cleavage of* $(Me_3S)PF_6$ *in the Presence of Proton Sources i-PrOH/i-PrOD or PrSH/PrSD*

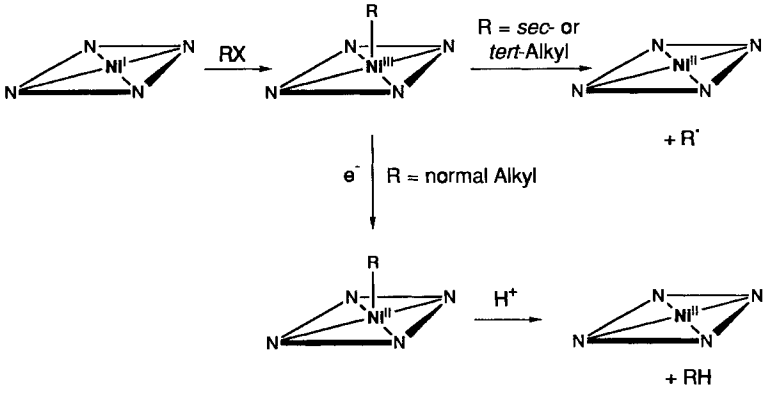
	i-PrOH/i-PrOD	PrSH/PrSD
Isotope effect on overall reaction rate[a]): k_H/k_D	2.8 ± 0.05	1.0 ± 0.05
Isotope effect on product distribution (competitive experiment)[b]): CH_4/CH_3D	11.0 ± 0.7	5.5 ● 0.3

[a]) k_H, methane-formation rate observed with the reaction system containing the pure ^{1}H-isotopomer; k_D, methane-formation rate observed with the reaction system containing the pure D-isotopomer.

[b]) Molar product ratio found in the reaction systems containing an equimolar mixture of the two isotopomers.

6. Discussion. – Two different mechanisms were proposed for the reduction of electrophiles, in particular of unbranched halides, by the Ni^I form of tetraaza-macrocyclic Ni complexes. *Gosden et al.* [11] interpreted the preferential formation of unbranched alkanes in the electrocatalytic reduction of normal alkyl halides as indicative of a mechanism where an oxidative addition is followed by one-electron reduction of the intermediate $[Ni^{III}(alkyl)]$ complex and protonolysis of the $[Ni^{II}(alkyl)]$ species according to *Scheme 6*. Mainly on the basis of the order of reactivity of primary, secondary, and

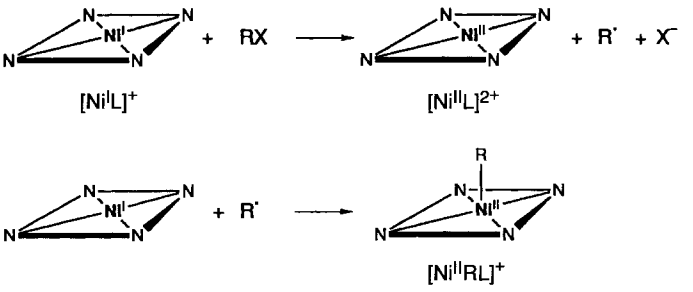
Scheme 6



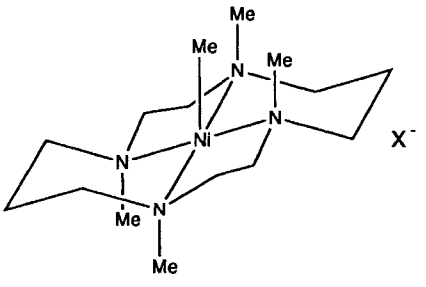
tertiary substrates, *Stoltzenberg* and *Stershic* [12] proposed the same mechanism for the reduction of normal alkyl halides by the Ni^{I} form of isobacteriochlorins[5]).

On the other hand, *Bakac* and *Espenson* [6] provided kinetic evidence pointing to a radical mechanism (*Scheme 7*) for the stoichiometric reduction of normal alkyl halides by [(*R,R,S,S*)-*N,N',N'',N'''*-tetramethylcyclam]nickel(I) ($[Ni^{I}(tmc)]^{+}$) in alkaline aqueous solution. Both mechanisms (*Schemes 6* and *7*) postulate the formation of a $[Ni^{II}(alkyl)]$ derivative of the Ni complex and subsequent protonation as the last steps of alkane formation. In fact, *Bakac* and *Espenson* [6] observed the absorption spectrum of a metastable species, which they attributed to $[Ni^{II}(methyl)(tmc)]^{+}$ (**3**), a compound which was first described by *D'Aniello* and *Barefield* [13] and recently characterized by NMR spectroscopy in our laboratory [14].

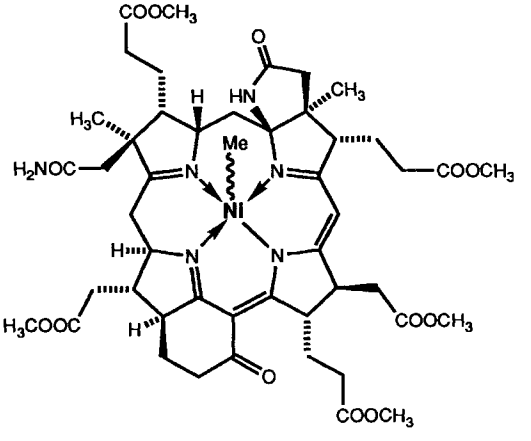
Scheme 7



Overall: $2[Ni^{I}L]^{+} + RX = [Ni^{II}RL]^{+} + X^{-} + [Ni^{II}L]^{2+}$



3 $[Ni^{II}(methyl)(tmc)]^{+}$



4 CH_3–F430M^{II}

[5]) Electrochemically generated $[Ni^{I}(oeibc)]$ (oeibc = octaethylisobacteriochlorin) in MeCN reacted with RX (X=Br, R=Me, Bu, *s*-Bu, *t*-Bu) in the order Me > Bu > *s*-Bu > *t*-Bu [12].

In the case of the reaction of F430M^{I} with sulfonium ions, both, the fact that the fourth H-entity is introduced into methane as a proton and the evidence for an intermediate species that is protonated to give methane (which follows from the isotope effects), point to the formation of a methylnickel(II) derivative **4** of F430M^{II} [14] as the last intermediate. In contrast to the much faster reaction of F430M^{I} with MeI [5], however, this intermediate did not accumulate in the reactions with sulfonium ions. A free-radical mechanism according to *Scheme 7* would be inconsistent with D-incorporation from i-PrOD as well as with the observed selectivity in the reduction of mixed dialkyl(methyl)sulfonium ions[6]). The preferential cleavage of Me-S over that of other alkyl–S bonds points to a close interaction between the Ni of F430M and the sulfonium ion in the product-determining step, consistent with a nucleophilic attack of Ni^{I} on the methyl C-atom as depicted in *Scheme 6*. However, the time-lag phenomenon and the very high apparent activation entropy both indicate that, in the case of the F430M-catalyzed reduction of sulfonium ions, the reaction sequence may be more complicated than depicted in *Scheme 6*. In contrast to the activation enthalpy, the numerical value calculated for the activation entropy from pseudo-first-order kinetic runs depends on the assumption that *Eqn. 1* is valid and that the active species is F430M^{I}. Although it was verified by UV/VIS spectroscopy that, within experimental error, F430M is quantitatively found in the Ni^{I} form during the entire kinetic run, the possibility that another species, formed from F430M^{I} in a preequilibrium and present in much lower concentration[7]), is attacking the sulfonium ion can not be excluded based on the available data. Further experimental work using more sensitive techniques, in particular EPR spectroscopy, is needed to test this hypothesis.

This work was supported by a research grant from ETH-Zürich. We thank Prof. *R. Thauer*, University of Marburg, Germany, for generous gifts of bacterial cells.

Experimental Part

1. *General. N,N*-Dimethylformamide (DMF; *Fluka*, for UV spectroscopy) was distilled at 40°/10 Torr over a 110-cm rectifying column with a reflux ratio of 8:1, rejecting the first 25% (*v/v*). The destillate was dried over molecular sieves (3 Å; freshly activated) for 2 days, decanted, and rectified once more, again rejecting the first 25%. DMF purified according to this method was stable for several weeks if stored under N_2 at −18°. (D_7)DMF (*Merck*, for NMR spectroscopy) was stored over molecular sieves (3 Å) for 2 days, decanted, and distilled at 40°/10 Torr. Dimethoxyethane (*Fluka, puriss.*) was freshly distilled from Na/benzophenone under N_2. Propanethiol (*Fluka, purum*) was distilled before use. Gas-solid adsorption chromatography: *Carlo Erba Fractovap G1* with flame-ionization detector and N_2 carrier gas; *Pyrex* column (2 mm i.d., 60 cm length) packed with *Porapak*, type *R*, 80–100 mesh (*Waters Associates, Inc.*) and activated overnight at 200°; separations at 100°; detection limit for methane < 1 nmol/ml; reproducibility for several injections of identical sample, ± 3%; identification of individual peaks by spiking with reference gases (*Matheson*, minimum purity 99.5%); retention times (t_R in min) and relative response factors (peak-height ratio *f*): methane (0.6, 1), ethene (1.0, 1.50), ethane (1.2, 1.40), propene (2.9, 2.12), propane (3.2, 2.53), 2-methylpropane (8.2, 0.937), and 2-methylpropene (9.5, 0.987). NMR-Spectra: *Bruker WM 300* (300 MHz) or *Varian-Gemini 200* (200 MHz) for ^{1}H; *Varian XL-300* (46.05 MHz), without lock, field drift < 1 Hz/h for

---

[6]) While, in view of the high bond-dissociation energy of H_2O, a mechanism according to *Scheme 6* may be plausible in H_2O, it would be unrealistic to postulate survival of a free Me radical, until it can react with a second molecule of F430M^{I} in MeCN or DMF.

[7]) If only 0.1% of the total catalyst would be present in this hypothetical active form, the calculated activation entropy would be reduced correspondingly to $-56\,J\,K^{-1}\,mol^{-1}$.

^2H; chemical shifts δ in ppm rel. to TMS, J in Hz. Mass spectra (m/z (rel.%)): *VG Tribrid* mass spectrometer, 70-eV electron-impact ionization, direct capillary inlet; GC/MS coupling of a *Finnigan* quadrupol mass spectrometer to a 20-m *SE30* capillary column at r.t.

2. *Identification of Gaseous Products by ^1H-NMR.* The top-space gas was sampled with a syringe and slowly passed through $CDCl_3$ or C_6D_6 in an NMR tube. ^1H-NMR ($CDCl_3$): methane: 0.216 (*s*); ethane: 0.855 (*s*); propane: 0.90 (*t*, $J = 7$, 6 H), 1.33 (*sept.*, $J = 7$, 2 H); ^1H-NMR (C_6D_6): propene 5.73 (*m*, 1 H), 4.99 (*dm*, 1 H), 4.98 (*m*, 1 H), 1.55 (*dd*, 3 H).

3. *Solubility of Methane in (D_7)DMF at 25°.* Under 1 atm of CH_4, 5.8 µmol/ml; under a partial pressure of 10^{-2} atm (typical reaction conditions), $5.5 \cdot 10^{-2}$ µmol/ml; determined by ^1H-NMR using C_6H_6 as internal standard.

4. *Deuterium-Labeled Compounds. (2-D)Propan-2-ol.* Acetone (*Fluka, puriss. p.a.;* 4 ml) was reacted with $NaBD_4$ (*Fluka,* > 99% D; 0.7 g, 14.6 mmol) in H_2O (4 ml) at 0° for 30 min. (2-D)Propan-2-ol was azeotropically distilled from the mixture, dried over two fresh portions of molecular sieves (3 Å), and distilled once more. ^1H-NMR ($CDCl_3$): 3.90 (*sept.*, < 0.01 H); 2.4 (br. *s*, 1 H); 1.24 (*s*, 6 H).

Propan-2-(D)ol. DCl (3.3M in D_2O; from *Fluka* 20% DCl, 99.5% D; 30 ml) was added dropwise at 0° to Al(i-PrO)$_3$ (*Fluka, pract.;* 7.87 g, 38.5 mmol). The propan-2-(D)ol was separated and purified as described above for (2-D)propan-2-ol. ^1H-NMR ($CDCl_3$): 4.01 (*sept.*, 1 H); 1.21 (*d*, 6 H); 1.45 (br. *s*, < 0.01 H, HDO, i-PrOH).

Propan-2-ol used in the competitive isotope-effect experiments was prepared from Al(i-PrO)$_3$ and HCl (3.3M in H_2O) using exactly the same procedure as for propan-2-(D)ol. ^1H-NMR ($CDCl_3$): 3.90 (*sept.*, *d*, 1 H); 2.38 (br. *d*, 1 H); 1.23 (*d*, 6 H).

Propane(D)thiol. Propanethiol (*Fluka, purum;* 1 ml, 11 mmol) was stirred with D_2O (*Fluka,* 99.8% D; 4 ml) under Ar for 1 h. The org. phase was separated and washed twice with 3 ml of D_2O. After collecting the org. phase, propane(D)thiol was filtered, dried ($NaCO_3$), and distilled. ^1H-NMR ($CDCl_3$): 2.48 (*t*, 2 H); 1.62 (*tq*, 2 H); 0.96 (*t*, 3 H); 1.31 (*t*, 0.045 H, residual SH).

Propanethiol used for competitive isotope-effect experiments was treated in the same way but with H_2O.

5. *Isotopomers of Methane for GC/MS Calibration. CH_4 (Matheson,* H.P., Grade 5). ^1H-NMR ($CDCl_3$): 0.216 (*s*). GC/MS (EI, 70 eV): 18 (< 1), 17 (2.2), 16 (100), 15 (74.3), 14 (30.3).

CD_3H. CD_3I (*Fluka,* 99.8% D; 1 ml) was stirred with conc. AcOH (1 ml) and Zn powder in a *Schlenk* ampule with septum under He (ionization in the MS was found to be insufficient in the presence of Ar). GC (molecular-sieves column, 100°): only methane. Calibration in GC/MS (*SE30* capillary column, 150 µl of 2 µmol methane/ml injected; EI, 70 eV): 19 (100), 18 (36.9), 17 (39.7), 16 (3.4).

CH_3D was prepared accordingly from CH_3I and (D_3)AcOD. ^1H-NMR ($CDCl_3$): 0.201 (*t* (1:1:1), $J = 1.8$, 3 H), 0.216 (*s*, 0.02 H, residual CH_4). GC/MS (EI, 70 eV): 18 (7), 17 (70.9), 16 (100), 15 (45.8), 14 (15.1).

CD_4 from CD_3I (*Ciba-Geigy,* > 99 atom-% D) and (D_3)AcOD. MS (EI, 70 eV): 20 (92.7), 19 (50.3), 18 (100), 17 (22.4), 16 (7.8).

6. *F430M^{II}* (**2**, X = ClO_4; M_r 1076.17) was prepared and purified according to [1c]. From kinetic runs, **2** was recovered as follows: Stirring of the amalgam was stopped, and after all F430M^I had reacted back to **2**, the soln. was transferred under N_2 to a flask containing 0.1M aq. $NaClO_4$/0.01M $HClO_4$. After repeated extraction with CH_2Cl_2, the combined org. phase was filtered and evaporated. The yield of recovery was determined by quant. UV/VIS and the purity checked by TLC or HPLC.

7. *Sulfonium salts* were synthesized according to [15] from the symmetric sulfides R_2S (*Fluka purum*, distilled) and trimethyloxonium hexafluorophosphate[8]) in CH_2Cl_2/MeCN 3:1. The yield of the raw product was nearly quantitative.

Trimethylsulfonium hexafluorophosphate was recrystallized twice from AcOEt. Yield 85%. ^1H-NMR (CD_3CN): 2.78 (*s*). ^1H-NMR ((D$_6$)acetone): 3.08 (*s*).

Diethyl(methyl)sulfonium hexafluorophosphate was recrystallized twice from AcOEt. Yield 66%. ^1H-NMR ((D$_6$)acetone): 3.50 (2*q*, 4 H); 3.02 (*s*, 3 H); 1.53 (*t*, 6 H).

(Methyl)dipropylsulfonium hexafluorophosphate was recrystallized twice from MeCN/1,2-dichloroethane. Yield 73%. ^1H-NMR ((D$_6$)acetone): 3.43 (*m*, 4 H); 3.02 (*s*, 3 H); 1.96 (*m*, 4 H); 1.12 (*m*, 6 H).

Diisopropyl(methyl)sulfonium hexafluorophosphate was recrystallized twice from acetone/EtOH. Yield 69%. ^1H-NMR (CD_2Cl_2): 3.67 (*m*, 2 H); 2.71 (*s*, 3 H); 1.56 (*d*, $J = 7$, 6 H); 1.53 (*d*, $J = 7$, 6 H).

---

[8]) We thank *A. Pfaltz* for a gift of $(Me_3O)PF_6$.

8. *Zinc Amalgam* (15% and 1.5%). A 15:85 (*w/w*) mixture of Zn powder (*Fluka, purum*) and Hg (*Merck*, anal. and polarography grade) in a test tube was topped by 10% aq. AcOH (5 ml) and heated to the boiling point while shaking vigorously. After the metal mixture had become homogeneous, the aq. phase was removed and the hot amalgam rinsed 5 times with H_2O. After cooling to r.t., the 15% Zn/Hg solidified and was stored under 2% aq. AcOH. Immediately before use, the amalgam was diluted by cutting an aliquot of the soft solid and adding 9 parts of Hg. The mixture was again heated to 100° under 10% aq. AcOH and shaken until it was homogeneous. It was washed several times with H_2O and with distilled MeOH and dried in a stream of N_2. The resulting 1.5% Zn/Hg was liquid and transferred to the reactors with a syringe.

9. *General Procedure for the F430M-Catalyzed Reduction of Sulfonium Ions.* The volume (*ca.* 18 ml) of the *Schlenk* reactor (*Fig. 4*) was determined by filling with H_2O and weighing. The sulfonium ion (typically 80–100 μmol) and $F430M^{II}$ (**2**; 0.5–1.0 μmol) in purified DMF (1.00 ml) were degassed in the reactor by three freeze-pump-thaw cycles, warmed to r.t., and flushed with Ar or He to 1 atm. In all reaction systems without formation of ethane, ethane (100 μl) was injected into the system as an internal reference to compensate for pressure changes and diffusion through the septum. The reaction was initiated by addition of 1.5% Zn/Hg (160 μl) and vigorous stirring. The soln. turned green ($F430M^{I}$, the Ni^{I} form of F430M) within 10–20 s. The reaction was monitored by GC analysis of 50-μl aliquots of top-space gas at regular intervals.

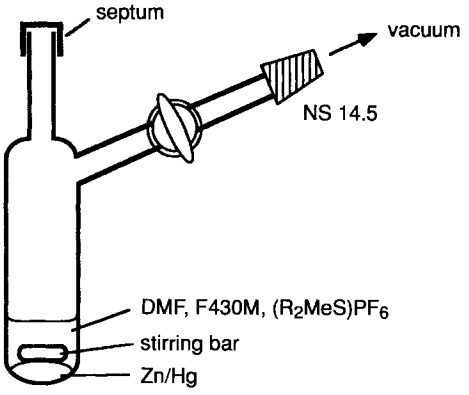


Fig. 4. Schlenk *reactor for the methane production*

Control reactions under identical conditions showed that from Me_2S (100 μmol) or from $(i\text{-}Pr)_2S$ (100 μmol), no propane, propene, or methane was produced (GC). In the absence of F430M, neither methane nor alkane nor alkene (GC) was formed from dialkyl(methyl)sulfonium hexafluorophosphates (alkyl = Me, Et, Pr, i-Pr). The control reactions were always run in parallel with the corresponding F430M-catalyzed reactions.

Dependence of the Kinetics of Methane Production on the Concentration of Propanethiol (*Fig. 1*). To four *Schlenk* reactors (*Fig. 4*), each containing F430M (1.00 μmol), $(Me_3S)PF_6$ (80 μmol), and DMF (1 ml), 250, 50, 10, and 2 μmol of propanethiol, respectively, were added. The reactions were started by addition of 200 μl of 1.5% Zn/Hg to each reactor; temp. 24.3 ± 0.5°.

Temperature Dependance of the Reaction Rate (*Fig. 2*; *Table 1*). Four parallel runs at −10, 0, 23, and 50° were carried out as follows: A soln. of F430M (1.954 mg; 1.816 μmol), $(Me_3S)PF_6$ (400 μmol), and propanethiol (400 μl) in DMF (4 ml) was equally divided into 4 reactors. After degassing and flushing with Ar, the reactors were immersed in liquid baths which were temp.-controlled to ± 0.1°. Then 1.5% Zn/Hg (200 μl) was added to every reactor and the reaction started by stirring. The results from the run at −10° had to be discarded because at −10° the amalgam was so viscous that quantitative steady-state reduction to $F430M^{I}$ could not be achieved as judged by the yellow-green color. Methane formation was followed by GC analysis calibrated against internal ethane as standard. After running for 3.5 h, totally 1.4 (0°), 6.8 (23°), and 32.4 μmol (50°) of methane were formed, respectively.

Deuterium Incorporation. Two parallel runs with DMF (1 ml), $(Me_3S)PF_6$ (88 μmol) and F430M (1 μmol) in each reactor. In addition, one reactor contained Me_2CDOH (100 μl), the other Me_2CHOD (100 μl); temp. 23°. The head-space gases were periodically analyzed by GC for 4 h. Then, the reaction was allowed to continue overnight.

The final composition of the head-space gas was analyzed by GC/MS (EI, 70 eV): system with Me_2CDOH, 12 μmol of CH_4 (MS: 17 (3), 16 (100)); system with Me_2CHOD, CH_3D/CH_4 85:15, 12 μmol (MS: 17 (12.5), 16 (11)).

Product Distribution from Mixed Sulfonium Salts (R₂MeS)PF₆. E.g.: [(i-Pr)$_2$MeS]PF_6 (27.82 mg, 100 μmol), F430M (1.00 mg; 0.929 μmol), and 1.5% Zn/Hg (160 μl) gave 8.34 μmol of methane (99.3%), 57 nmol of propane (0.7%), and 62 nmol of propene, after stirring for 260 min. Within exper. error, the product distribution remained the same during the whole run.

Isotope Effect on the Rate of Methane Formation in Parallel Reactions. a) *Propan-2-ol* (*Fig. 3a, Table 3*). F430M (1.113 mg) and $(Me_3S)PF_6$ (49.4 mg, 226 μmol; dried *in vacuo*) were dissolved in DMF (2.27 ml). This soln. (1.00 ml) and i-PrOH (50 μl; *Fluka, puriss. p.a.*, distilled) or i-PrOD (50 μl) were transferred to 2 reactors; temp. 24°. The head-space gas was analyzed by GC (ethane as internal standard). *i-PrOH Exper.* (*t* [min] (CH_4 [μmol])): 32 (0.17), 51 (0.40), 64 (0.58), 81 (0.94), 102 (1.41), 143 (2.55). *i-PrOD Exper.* (*t* [min] (CH_3D [μmol])): 35 (0.063), 52 (0.134), 65 (0.192), 77 (0.29), 104 (0.49), 144 (0.92). This experiment was repeated twice and gave the same results (within the error indicated in *Table 3*).

b) *Propanethiol* (*Fig. 3b, Table 3*). As described above for propan-2-ol, but with PrSH (50 μl) and PrSD (50 μl), resp., in the two reactors; temp. 20.5°. *PrSH Exper.* (*t* [min] (CH_4 [μmol])): 6.5 (0.32), 14 (0.83), 24.5 (1.46), 43 (2.47), 55 (3.17), 70 (3.89), 95 (5.22), 106 (5.73), 128 (7.19), 153 (7.85). *PrSD Exper.* (*t* [min] (CH_3D [μmol])): 6.5, (0.29), 14 (0.77), 23 (1.33), 42.5 (2.25), 55.5 (2.85), 70 (3.52), 95 (5.01), 105.5 (5.95), 129 (6.19), 153 (6.82). The D-content of the resulting methane was checked by ^{1}H-NMR ($CDCl_3$) as described above and gave (*PrSD Exper.*): 0.201 (*t*, CH_3D), 0.216 (*s*, CH_4), CH_3D/CH_4 85:15.

Competitive Reaction with 1:1 Mixtures of the Two Isotopomers. Propan-2-ol, Propanethiol (*Table 3*). F430M (1.00 mg) and $(Me_3S)PF_6$ (44.43 mg; 200 μmol) were dissolved in DMF (2.00 ml). The soln. was divided into 2 reactors, one containing i-PrOH/i-PrOD 1:1 (50 μl), the other PrSH/PrSD 1:1 (50 μl). The degassed reaction systems were stirred with 1.5% Zn/Hg (160 μl). The head-space gas was analyzed by GC/MS after 7, 13, and 19% conversion. Finally (at 20% conversion), the head-space gas was passed through $CDCl_3$ in an NMR tube and the CH_4/CH_3D ratio determined by ^{1}H-NMR. Both GC/MS and ^{1}H-NMR gave ratios CH_4/CH_3D of 11.0 ± 0.7 (system with propan-2-ol) and 5.5 ± 0.3 (system with propanethiol; see *Table 3*). No significant variation of the isotopic composition at different conversions was detectable.

10. *Reductive Cleavage of Mixed Sulfonium Ions with Sodium Naphthalide.* Dialkyl(methyl)sulfonium hexafluorophosphates (*ca.* 100 μmol) were dissolved under Ar in dimethoxyethane (DME; 0.500 ml) and degassed by three freeze-pump-thaw cycles. A 50 mM sodium naphthalide soln. (prepared by reduction of naphthalene (32.1 mg) with Na granulate in DME (5 ml)); 1 ml was added under Ar at 22.5°. As judged by the immediate disappearance of the dark green color of sodium naphthalide, the reaction was practically instantaneous. GC analysis of the head-space gas yielded: from $(Et_2MeS)PF_6$ (26 mg, 104 μmol): methane (1.37 μmol), ethene (1.77 μmol), ethane (1.77 μmol), and propane (0.102 μmol); from $(Pr_2MeS)PF_6$ (27.3 mg, 98 μmol): methane (1.22 μmol), propene (0.41 μmol), and propane (1.38 μmol); from [(i-Pr)$_2$MeS]PF_6 (27.6 mg, 99.3 μmol): methane (0.15 μmol), propene (1.38 μmol), and propane (0.29 μmol).

Control reactions under identical conditions showed that from Me_2S (100 μmol) or (i-Pr)$_2$S (100 μmol), no propane, propene, or methane was produced (GC), while the color of sodium naphthalide persisted for more than 30 min.

REFERENCES

[1] a) R.P. Gunsalus, R.S. Wolfe, *FEMS Microbiol. Lett.* **1978,** *3,* 191; b) G. Diekert, B. Klee, R.K. Thauer, *Arch. Microbiol.* **1980,** *124,* 103; c) A. Pfaltz, B. Jaun, A. Fässler, A. Eschenmoser, R. Jaenchen, H.H. Gilles, G. Diekert, R.K. Thauer, *Helv. Chim. Acta* **1982,** *65,* 828; d) D.A. Livingston, A. Pfaltz, J. Schreiber, A. Eschenmoser, D. Ankel-Fuchs, J. Moll, R. Jaenchen, R. K. Thauer, *ibid.* **1984,** *67,* 334; e) A. Pfaltz, D.A. Livingston, B. Jaun, G. Diekert, R.K. Thauer, A. Eschenmoser, *ibid.* **1985,** *68,* 1338; f) A. Pfaltz, 'Structure and Properties of Coenzyme F430', in 'The Bioinorganic Chemistry of Nickel', Ed. J.R. Lancaster, VCH Publishers, New York, 1988, pp. 275–298; g) G. Färber, W. Keller, Ch. Kratky, B. Jaun, A. Pfaltz, Ch. Spinner, A. Kobelt, A. Eschenmoser, *Helv. Chim. Acta* **1991,** *74,* 697.

[2] a) J. Ellermann, S. Rospert, R.K. Thauer, M. Bokranz, A. Klein, M. Voges, A. Berkessel, *Eur. J. Biochem.* **1989,** *184,* 63; b) W.L. Ellefson, W.B. Whitman, R.S. Wolfe, *Proc. Natl. Acad. Sci. U.S.A.* **1982,** *79,* 3707; c) K.M. Noll, K.L. Rinehardt, Jr., R.S. Tanner, R.S. Wolfe, *ibid.* **1986,** *83,* 4238; d) K.M. Noll, M.I. Donelly, R.S. Wolfe, *J. Biol. Chem.* **1987,** *262,* 513; e) A. Kobelt, A. Pfaltz, D. Ankel-Fuchs, R.K. Thauer,

FEBS Lett. **1987,** *214,* 265; f) T.A. Bobik, K.D. Olson, K.M. Noll, R.S. Wolfe, *Biochem. Biophys. Res. Commun.* **1987,** *149,* 455; g) J. Ellermann, R. Hedderich, R. Böcher, R.K. Thauer, *Eur. J. Biochem.* **1988,** *172,* 669.

[3] S. Rospert, R. Böcher, S.P.J. Albracht, R.K. Thauer, *FEBS Lett.* **1991,** *291,* 371.

[4] B. Jaun, A. Pfaltz, *J. Chem. Soc., Chem. Commun.* **1986,** 1327.

[5] B. Jaun, A. Pfaltz, *J. Chem. Soc., Chem. Commun.* **1988,** 293.

[6] A. Bakac, J.H. Espenson, *J. Am. Chem. Soc.* **1986,** *108,* 713.

[7] K.W. Egger, A.T. Cocks, *Helv. Chim. Acta* **1973,** *56,* 1516.

[8] B. Blank, A. Henne, G.P. Laroff, H. Fischer, *Pure Appl. Chem.* **1974,** *41,* 475; J.K. Thomas, *J. Phys. Chem.* **1967,** *71,* 1919.

[9] J. Grimshaw, 'Electrochemistry of Sulphonium Groups', in 'The Chemistry of the Sulphonium Group', Ed. C.J.M. Stirling, Wiley, New York, 1981, p. 141; J.Q. Chambers, 'Organic Sulfur Compounds', in 'Encyclopedia of Electrochemistry of the Elements', Section XII-3, Eds. A.J. Bard and H. Lund, Dekker, New York, 1978, p. 476; A. Luettrighaus, H. Machatzke, *Ann. Chem.* **1964,** *671,* 165.

[10] T. Wataki, M. Miyoshi, M. Matsuoka, K. Matsumoto, *Chem. Ind. (London)* **1973,** 1163.

[11] C. Gosden, K.P. Healy, D. Pletcher, *J. Chem. Soc., Dalton Trans.* **1978,** 972.

[12] A.M. Stolzenberg, M.T. Stershic, *J. Am. Chem. Soc.* **1988,** *110,* 5397.

[13] M.J. D'Aniello, Jr., E.K. Barefield, *J. Am. Chem. Soc.* **1976,** *98,* 1610.

[14] S.-K. Lin, B. Jaun, *Helv. Chim. Acta* **1991,** *74,* 1725.

[15] D. Klamann, Ed., 'Organische Schwefelverbindungen', in 'Houben-Weyl, Methoden der organischen Chemie', Band E11, 4. Aufl., Thieme, Stuttgart, 1985, Teil 1, S. 418.