The Chemical Evolution of a Nitrogenase Model, 20
Alkyl Molybdates(VI) and the Mechanism of Hydrocarbon Production
by Nitrogenase [1]

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Nitrogenase Model, Alkyl Molybdates

Colorless alkylmolybdates(VI) of composition R-M0O3- are generated in aqueous solutions by the alkaline hydrolysis of complexes R-Mo(Bpy)2Br (Bpy = 2,2'-bipyridyl, R = CH3 and higher alkyl). At room temperature in alkaline aqueous solution, the new organometallic derivatives of oxomolybdate(VI) are remarkably resistant against Mo-C bond hydrolysis. Decomposition occurs more rapidly on heating, affording unrearranged alkanes according to the eq.: R-M0O3- + OH- → RH + MoO4=.

In acidic solutions, the methylmolybdate(VI) species decomposes with the formation of a mixture of methane and ethane while higher alkylmolybdates carrying hydrogen in the β-position relative to molybdenum undergo Mo-C bond heterolysis by way of β-elimination: R-CH2CH2-M0O3- → Mo4(aq) + H+ + R-CH = CH2.

The Mo-C bond of alkylmolybdates is resistant to oxidants but is very sensitive to cleavage under reducing conditions. Reductive Mo-C bond cleavage occurs particularly rapidly in the presence of thiols and reduced ferredoxin model compounds. The latter reactions simulate the terminal steps of hydrocarbon producing reactions of nitrogenase with alternate substrates such as CN-, R-CN or R-NC, confirming previous mechanistic conclusions concerning the mechanism of nitrogenase action.

Introduction

In paper XVI of this series we reported the synthesis of novel organomolybdenum(VI) complexes of Type I (with R = CH3 and C2H5) and showed that they react with cold aqueous alkali without extensive Mo-C bond cleavage [2]. This suggested that intermediate anionic "alkylmolybdate(VI)"-species, R-M0O3- and related organometallic derivatives of oxomolybdate(VI) are capable of existence. We have since investigated the reactions of these intriguing new organometallic ions in greater detail. In addition to studies of Mo-C bond hydrolysis, the reductive cleavage of the Mo-C bond in these species was investigated as well, since previous studies [3-5] indicated that the reductive cleavage of the Mo-C bond of enzymic alkylmolybdenum intermediates occurs in the terminal step of hydrocarbon producing reactions of nitrogenase with substrates such as CN-, R-CN or R-NC.

Results

Preparation and properties of alkylmolybdates in solution

In cold, dilute ammonium hydroxide solution complexes of Type I undergo hydrolysis to alkyl molybdates of Type III via intermediate red complexes of Type II (eq. (1)).

After removal of the Bpy released during the reaction, colorless solutions are obtained which decompose with the formation of alkanes according to eq. (2) (Fig. 1),

\[
\begin{align*}
\text{R-MoO}_3^- + \text{H}_2\text{O} \rightarrow & \text{RH} + \text{MoO}_4\text{H}^- \\
\text{II} \rightarrow \text{III} \rightarrow \text{IV}
\end{align*}
\]
causing solutions of all alkylmolybdates to contain variable amounts of molybdate. The presence of the alkylmolybdenum species in solution follows unambiguously from the $^1$H NMR spectra of solutions of the alkylmolybdates in D$_2$O (Table I). The chemical shift of the signal of the protons of CH$_3$MoO$_3^-$ changes little in the pH range of 4.0-12. It disappears slowly on standing, more rapidly on heating of the solutions, with concomitant formation of CH$_3$D.

Table I. Proton NMR shifts for alkyl-molybdates, R-MoO$_3$-

<table>
<thead>
<tr>
<th>R</th>
<th>Chemical shifts in ppm and integration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$-\text{CH}_3$</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>0.611(1) s</td>
</tr>
<tr>
<td>C$_2$H$_5$</td>
<td>2.0(3)t</td>
</tr>
<tr>
<td>n-C$_3$H$_7$</td>
<td>2.0(3)t</td>
</tr>
<tr>
<td>t-C$_3$H$_7$</td>
<td>2.1(6)d</td>
</tr>
<tr>
<td>t-C$_4$H$_9$</td>
<td>2.3(9)s</td>
</tr>
<tr>
<td>$-\text{CH}_2$-$\text{C}$(CH$_3$)$_3$</td>
<td>2.1(9)s</td>
</tr>
</tbody>
</table>

The spectra were measured on a Nicolet Fourier transform 360 MHz NMR in neutral D$_2$O solution. All spectra were at 23 degrees Centigrade with TMS as the internal standard.

Alkaline hydrolysis of the Mo–C bond

The rates of decomposition increase with increasing alkalinity of the solutions. For methylmolybdate at room temperature in 0.1 to 1 M NaOH, a first order dependence of the rate of methane evolution on OH$^-$ was observed (see Table II).

Table II. Base dependence of methane evolution with methyl-molybdate at 0 °C.

<table>
<thead>
<tr>
<th>[NaOH]</th>
<th>k (min$^{-1}$)</th>
<th>t$_{1/2}$ [h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 N</td>
<td>$4.4 \times 10^{-4}$</td>
<td>26.5</td>
</tr>
<tr>
<td>0.2 N</td>
<td>$7.0 \times 10^{-4}$</td>
<td>16.5</td>
</tr>
<tr>
<td>0.4 N</td>
<td>$13.0 \times 10^{-4}$</td>
<td>8.9</td>
</tr>
<tr>
<td>0.8 N</td>
<td>$27.0 \times 10^{-4}$</td>
<td>4.3</td>
</tr>
</tbody>
</table>

The Arrhenius energies of activation of Mo–C bond hydrolysis for methylmolybdate (to methane) and of ethylmolybdate (to ethane) were determined to be 17 kcal/mole in the temperature range between 0 and 70 °C (experimental data are shown in Fig. 1).

Hydrolysis in neutral and acidic solutions

The formation of alkanes from alkylmolybdates also occurs in neutral and weakly acidic solutions, albeit at significantly slower rates than in alkaline media. Observed rates and t$_{1/2}$ times of decomposition for the alkylmolybdenium species of the present study are summarized in Table III.

Table III. Half-lives and product distribution of aqueous R-molybdates at 70 °C.

<table>
<thead>
<tr>
<th>pH 11$^a$</th>
<th>pH 1.3$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-</td>
<td>t$_{1/2}$</td>
</tr>
<tr>
<td>R-</td>
<td>[min]</td>
</tr>
<tr>
<td>methyl</td>
<td>60</td>
</tr>
<tr>
<td>ethyl</td>
<td>29.3</td>
</tr>
<tr>
<td>n-propyl</td>
<td>34.3</td>
</tr>
</tbody>
</table>
t-propyl   | 40.8      | 13.2   | 4.6       | 0.07    |
t-butyl    | 96.0      | 1.0    | 14.0      | isobutene |
|neopentyl  | 282.0     | neopentane | 16.2 | neopentane |

$^a$ Done in pH 11 .1 N phosphate buffer; $^b$ done in .1 N HCl.
While methane is the only product of Mo-C bond hydrolysis of methyl molybdate species in dilute mineral acids, increasing amounts of ethane are formed on hydrolysis in concentrated acids. In 3 N HCl, or 2 N H₃PO₄, for example, about 3:1 mixtures of methane and ethane are generated and Mo(VI) is reduced. A pH-hydrocarbon profile of the decomposition of methylmolybdate is shown in Fig. 2.

Higher alkylmolybdates carrying hydrogen in β-position relative to molybdenum decompose in acidic solution predominantly by way of olefin formation. The pH profile of hydrocarbon production for ethylmolybdate is shown in Fig. 3. It may be seen that ethylene becomes the main hydrocarbon product of decomposition in acidic solutions below the pH of 2. The Arrhenius energy of activation for the decomposition of ethylmolybdate to ethylene in acidic solutions was determined to be 9 kcal/mole in the temperature range between 0 and 70 °C and thus is significantly lower than that observed for its decomposition to ethane in alkaline solutions.

Isolation of alkylmolybdate species in the solid state

Since alkali- and alkaline earth metal salts of alkylmolydbatic acids are water soluble, neutral or mildly alkaline aqueous solutions of methylmolybdate were evaporated to dryness in vacuo to obtain them in the solid state. Although significant amounts of methane evolved from the solutions during evaporation, the colorless residues which were obtained still contained Mo–CH₃ groups as evidenced by the formation of significant amounts of CH₄ on heating in 1 M NaOH. However, even after drying in a high vacuum, the solids proved unstable on storage and continued to decompose with methane evolution; a similar behavior was also observed with the solids precipitating from solutions of methyl molybdate upon the addition of acetone.

On careful evaporation of acidic solutions (pH 2, HCl) of methyl molybdate, methane is also evolved. The amorphous solids which were ultimately isolated, decomposed on storage, losing ca. 10% of the methyl groups present every 24 h. The solids contain presumably polymeric methylmolybdic acids since they proved to be nearly insoluble in water. They are freely soluble in dilute aqueous NaOH, however. Evidence for the presence of Mo–CH₃ species in these solutions was obtained by way of a methyl group transfer experiment in terms of eq. (3):

$$\text{R}^+\text{Mo}^{5+}\rightarrow\text{R}^+[\text{Mo}^{6+}]$$

Oxidative Mo–C bond cleavage

Alkylmolybdates appear to be resistant to attack by oxidants such as H₂O₂, dil. HNO₃, MnO₄⁻,
alkylmolybdate, a thiol ligand such as cysteine, ferredoxin model compound (preformed or generated in situ), and a reducing agent (Na$_2$S$_2$O$_4$). In Fig. 4, the relative rates of methane production from methyl molybdat e are plotted against the charge $z$ of the anions [Fe$_4$S$_4$(SR)$_4$]$^{2-}$. It may be seen that the methane production increases linearly with $z$, suggesting that Mo–C bond cleavage occurs by way of a one electron process. This result prompted us to explore the reductive cleavage of the Mo–C bond of methylmolybdate with V$^{3+}$(aq), another one-electron reductant. At pH 7 in homogeneous solution, a five-fold enhancement of methane production was observed.

**Discussion**

Alkylmolybdates are novel organometallic derivatives of oxomolybdates(VI). They are of particular interest because of the relative resistance of the Mo–C bond to hydrolysis and the carbanionic reactivity of the molybdenum-bound alkyl residues to hydrolysis in protic media.

The colorless anions R–MoO$_3^-$ are the predominant species in neutral and alkaline solutions. Their decomposition in alkaline media according to eq. (2) may be assumed to occur by way of an intermediate dianion such as IV, which undergoes further reaction according to eq. (4):

\[ \text{alkylmolybdate + thiol ligand + ferredoxin model compound + reducing agent} \rightarrow \text{hydrocarbon + products} \]
The relative resistance of the Mo–C bond to alkaline hydrolysis is attributed to the high partial negative charge density on molybdenum which renders hydrolysis in terms of eqs. (2) or (4) the only energetically favored pathway of decomposition in alkaline solutions.

In neutral and weakly acidic solutions (pH 7–2), the anions R–MoO₃⁻ are still the predominant species in solution since the hypothetical acids R–MoO₂H are expected to be completely dissociated in this pH range. Accordingly, it is not surprising that alkanes are also the major products of Mo–C bond hydrolysis under these conditions. The free alkylmolybdcic acids or their alkali salts as isolated from aqueous solutions are unstable on storage, decomposing with hydrocarbon evolution. This is because the solids still contain tightly bound water which furnishes the protons required for Mo–C bond hydrolysis. Anhydrous salts of alkylmolybdcic acids should be stable, but thus far have not become available. In concentrated acids, the ions R–MoO₂⁺ are probably formed. They are also unstable in solution and decompose spontaneously with hydrocarbon evolution. Because of the net positive charge on molybdenum in these ions, a transfer of electrons from the alkyl group is possible, giving rise to the formation of olefins and reduced molybdenum species as exemplified in eq. (5) for the ethylmolybdenum derivative:

\[
\begin{align*}
\text{C}_2\text{H}_5\text{CH}_3 & \quad \text{Mo}^+(\text{aq}) + \text{H}^+ + \text{C}_3\text{H}_6 \\
\end{align*}
\]

Experimental
Reagents, chemicals and stock solutions
All chemicals and reagents were of either “analytical” or “reagent” grade purity and were used as received.

Preparation of alkylmolybdates in solution
The alkylmolybdates were generated in solution through the careful hydrolysis of complexes R–Mo(bpy)(O)₃Br. The latter were prepared from the reactions of Mo(bpy)(O)₃Br₂ with organomagnesium halides in tetrahydrofuran as described in ref. [2], i.e. with R = CH₃, C₂H₅, n-C₃H₇, i-C₃H₇, -C(CH₃)₃, -CH₂–C(CH₃)₃. To generate the solutions of the alkylmolybdates the following general method was used:

Into a beaker of 250 ml capacity, 1 g of R–Mo(bpy)(O)₃Br₂ was suspended in 100 ml of deionized water. To the slurry, 1 ml of 6 N NH₄OH solution was added and the mixture was magnetically stirred at room temperature for 5 min. The reaction mixture turns red-brown due to the intermediate formation of labile complexes of Type II. After 5 min, the solution was cooled with ice; colorless crystals of bipyridyl are formed at this point which are removed by suction filtration. Small amounts of Bpy remaining in the solution are removed from

Biochemical implications
The model studies have shown that all substrate reactions of nitrogenase occur at a mononuclear molybdenum active site situated in a sterically hindered (“pocketed”) environment. They also indicated that non-heme iron clusters participate in the reduction of the substrates only indirectly by catalyzing the transfer electrons from the external reductant to the molybdenum active site. Since the molybdenum atom of nitrogenase is generally assumed to be attached to a protein-S⁻ residue, the complexes formed on interaction of alkyl molybdates with thiols qualify as models of alkylated intermediates which are believed to be formed during the reduction of substrates such as CN⁻, R–NC and R–CN. Our present work shows that the reactions of such intermediates can be studied directly by generating complexes of alkylmolybdates with thiols in situ. As expected, the Mo–C bond in these complexes is more susceptible to cleavage than in the simple alkyl molybdates themselves. However, the reductive Mo–C bond cleavage occurs with highest efficiency in the “complete” nitrogenase model systems, i.e. in the presence of the reduced ferredoxin clusters. Under the same conditions, catalytic as well as stoichiometric reductions of acetylene also proceed with maximum efficiency. Hence, the behavior of the alkylmolybdenum species observed in the present work is fully compatible with that postulated [3–5] for the hypothetical organomolybdenum intermediates in certain catalytic reactions of nitrogenase and the molybdothiol model systems of nitrogenase.
the filtrate by extraction with CH$_2$Cl$_2$. The resulting solutions are initially faintly yellow but become colorless after a few minutes of standing. For analysis, 1 ml samples of the solutions are placed into reaction vials of 38 ml capacity which are sealed with silicon serum caps. To these, 5 ml of 25% NaOH are injected by means of a syringe. The vials are then placed into an oven and kept at 80 °C for 1 h. The yields of hydrocarbons are measured by means of gas-liquid-phase chromatography at 27 °C (or higher operating temperatures where necessary), using a 6 ft column filled with 80-100 mesh phenylisocyanate-Porasil C, with helium as the carrier gas, and a flame ionization detector. The solutions of the more stable methyl- and neopentylmolybdates can be stored for several days at 0-5 °C with only insignificant Mo-C bond hydrolysis occurring; it is advisable to prepare fresh solutions of the less stable higher n-alkylmolybdates immediately prior to the experiments.

**Mo–C bond cleavage experiments with ferredoxin model compounds**

The Mo–C bond cleavage experiments were usually carried out in silicon serum capped bottles of 38 ccm capacity; details of the experimental conditions are given in the legends to Figures and Tables. Crystal-line salts of the ferredoxin model compounds, i.e. N(n-C$_4$H$_9$)$_4$[$\text{Fe}_4\text{S}_4\text{(RS)}_4]^-$ (with R = n-C$_3$H$_7$) were synthesized as described in ref. [8] l.c.; the preparation of the [Fe$_8$S$_4$(RS)$_4]^-$ (z = 2-4) was described in ref. [6].

**Alkyl group transfer experiment**

A weakly acidic solution of methylmolybdate (pH 2) was evaporated in vacuo. The resulting gray-blue solid (50 mg) was dissolved in 1 ml of pH 9, 0.2 N phosphate buffer. To this was added 5 ml of a freshly prepared solution of 5 mg of hydroxocobalamin in 0.2 N pH 9 phosphate buffer. After 1 h of reaction in the dark at room temperature, the corrins were removed by phenol extraction and isolated in the solid state by the addition of acetone and diethyl ether. Methylcobalamin was identified on the basis of its optical absorption spectrum, behavior on photolysis and its Rf value as described in ref. [2].

**Reductive Mo–C bond cleavage with V$^{+3}$ (aq)**

Alicuots (2 ml) of a freshly prepared solution of methylmolybdate in pH 7.2 0.1 N phosphate buffer were placed in serum-capped glass vials flushed with Ar. To one vial, 1 ml of a 1 M, pH 7 buffered VCl$_3$ solution was added. After 30 min the rel. yields of CH$_4$ were determined by glpc.

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