Studies on Vitamin B$_{12}$ and Related Compounds, 51 [1]

Direct Syntheses of Alkylcobalamins from Alkanes and Vitamin B$_{12r}$ under “Oxidizing-Reducing” Conditions

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Syntheses of methylcobalamin from methane, and of n-alkylcobalamins from n-alkanes (C$_2$→C$_{10}$) and vitamin B$_{12r}$ are described. The compounds are formed under “oxidizing-reducing” conditions: Oxygen radicals (O$_2$, HO-, and HO$_2^-$) are generated from the reaction of O$_2$ with reducing metal ions, notably V$^{+3}$(aq) and abstract hydrogen from the alkane substrates. The resulting alkyl radicals are captured by vitamin B$_{12r}$ with high efficiency to yield alkylcobalamins. The reactions take place at room temperature in mildly alkaline or acidic solutions. In addition to n-alkylcobalamins, the preparation of neopentylecobalamin from neopentane, of isobutylecobalamin from isobutane, and of several cycloalkylecobalamins from cycloalkanes, is also reported. Methyl radicals generated in D$_2$O from CH$_4$ under “oxidizing-reducing” conditions are reduced to yield CH$_3$D.

Until recently, all methods of synthesis of organocobalamins were based on reactions of vitamin B$_{12r}$ derivatives with functionalized unsaturated or otherwise activated organic compounds as the substrates. In two previous papers of this series [1, 2], a new method of synthesis of organocobalamins was described which utilizes normal, non-activated organic substrates as the substrates. In this method, organic radicals are generated from the substrates through reactions with oxygen radicals. The latter are formed from molecular oxygen or H$_2$O$_2$ on reaction with reducing metal ions, usually V$^{+3}$(aq). The organic radicals generated by H-abstraction are then captured by vitamin B$_{12r}$

([Co$^{+2}$]) to yield the organocobalamins ([Co]) according to reaction eq 1:

\[
R-H \rightarrow [H] \rightarrow R- \rightarrow [Co] + [Co^{+2}] \rightarrow R-[Co]
\]

(1)

The reducing metal ion, i.e. V$^{+3}$(aq), plays the important role of reducing molecular oxygen to oxygen radicals and of vitamin B$_{12a}$ (hydroxocobalamin) to vitamin B$_{12r}$. It is applied in excess relative to the oxidant to assure the maintenance of the vitamin in the reduced (Co$^{+2}$-) state. In this manner a wide variety of organocobalamins has become accessible from diverse substrates such as carboxylic acids [2], aldehydes, alcohols, ethers [1], etc. It therefore seemed logical to expect that even alkanes could be used as the substrates for the synthesis of alkylcobalamins under “oxidizing-reducing” (O/R-) conditions. Attempts to verify such reactions have been successful and will be described in the following.

Results

Methylcobalamin from methane

Methyl radicals generated according to reaction eq (1) react with vitamin B$_{12r}$ to form methylcobalamin, but at 1 atm of CH$_4$ pressure the yields are low due to the poor water solubility of CH$_4$. At 3 atm of CH$_4$ pressure, yields of methylcobalamin increased to 40% (based on total vitamin B$_{12}$ employed). The methylcobalamin was isolated from the reaction solutions and identified by thin-layer chromatography, spectroscopic measurements and analysis of the hydrocarbon products formed on photolysis (see Experimental section).

To demonstrate that CH$_4$ is converted to CH$_3$ radicals under O/R conditions, solutions of V$^{+3}$(aq) in D$_2$O were kept in contact with CH$_4$ gas at 1 atm in the presence of oxygen as well as under strictly
Table I. Yields and Rf values of alkylcobalamins from reactions of vitamin B12r with alkanes under oxidizing/reducing conditions employing V(III) aq. as the reductant and O2 as the oxidant at 25 °C.

<table>
<thead>
<tr>
<th>Alkane substrate</th>
<th>Product R</th>
<th>Rf values</th>
<th>Yields^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH4 (1 atm)</td>
<td>R = CH3</td>
<td>.43</td>
<td>.63</td>
</tr>
<tr>
<td>(3 atm)</td>
<td>CH3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2H6 (1 atm)</td>
<td>C2H5</td>
<td>.46</td>
<td>.64</td>
</tr>
<tr>
<td>(2 atm)</td>
<td>C2H5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H8 (1 atm)</td>
<td>n-C3H7</td>
<td>.50</td>
<td>.65</td>
</tr>
<tr>
<td>(2 atm)</td>
<td>n-C3H7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-C4H10 (2 atm)</td>
<td>n-C4H9</td>
<td>.53</td>
<td>.67</td>
</tr>
<tr>
<td>n-C5H12 (0.5 ml liqu.)</td>
<td>n-C5H11</td>
<td>.57</td>
<td>.69</td>
</tr>
<tr>
<td>n-C6H14 (0.5 ml liqu.)</td>
<td>n-C6H12</td>
<td>.60</td>
<td>.70</td>
</tr>
<tr>
<td>n-C7H16 (0.5 ml liqu.)</td>
<td>n-C7H15</td>
<td>.63</td>
<td>.71</td>
</tr>
<tr>
<td>n-C8H18 (0.5 ml liqu.)</td>
<td>n-C8H17</td>
<td>.65</td>
<td>.73</td>
</tr>
<tr>
<td>n-C9H20 (0.5 ml liqu.)</td>
<td>n-C9H19</td>
<td>.68</td>
<td>.73</td>
</tr>
<tr>
<td>n-C10H22 (0.5 ml liqu.)</td>
<td>n-C10H21</td>
<td>.64</td>
<td>.72</td>
</tr>
</tbody>
</table>

^a TLC on cellulose in the following solvent systems: (I) Water saturated 2-butanol; (II) n-butanol-isopropanol:water, 7:6:7 (vol.); (III) n-butanol:ethanol:water, 10:3:7 (vol.).

^b Based on total vitamin B12r employed. Yields determined spectrophotometrically and by TLC.

^c Mixed by ultrasonication.

anaerobic conditions. Anaerobically, no evidence for the formation of deuteriated methane derivatives was obtained even after 24 h. However, in the presence of small amounts of oxygen, CH3D became detectable mass-spectrographically after about 2 h of reaction. The formation of CH3D is caused through the reduction of CH3 radicals by V+3(aq) and their subsequent reaction with D+ under the reaction conditions.

Higher n-alkylcobalamins from alkanes

A representative number of higher n-alkylcobalamins was prepared from reactions of straight-chain alkanes with vitamin B12r under O/R conditions. Table I shows that the yields of the lower n-alkylcobalamins increase with increasing pressure of gaseous alkanes; the highest yields were obtained with n-butane as the substrate. With liquid alkanes, the yields were below 5%. A substantial improvement of the yields of alkylcobalamins was achieved by ultrasonication of the heterogeneous reaction mixtures. Hence, it is evident that the low solubility of the hydrocarbons in the reaction solutions was chiefly responsible for the poor yields. All n-alkylcobalamins were isolated and identified by comparison with the respective cobalamins prepared by conventional methods of synthesis. By conducting the reaction under acidic conditions, attempts were made to detect the formation of sec.-alkylcobalamins in a number of cases. With propane as the substrate, evidence for the formation of some isopropylcobalamin was obtained, but this cobalamin was not isolated because of its instability in neutral aqueous solution.

Cycloalkylcobalamins from cycloalkanes

Cyclopropyl-, cyclopentyl-, and cyclohexylcobalamin were obtained by the reactions of the corresponding cycloalkanes with vitamin B12r under O/R conditions in acidic solutions (0.1 M HCl). Acidic reaction conditions were chosen to prevent the decomposition of the cycloalkylcobalamins. The cobalamins were identified by analysis of the gaseous photolysis products and comparison with authentic samples of cycloalkylcobalamins synthesized as has been described elsewhere [3]. The yields of cyclopropyl- and cyclopentylcobalamin were 54 and 66%, respectively. Cyclohexylcobalamin was obtained with 19% yield, based on total vitamin B12.

Neopentylcobalamin and isobutylcobalamin

Neopentylcobalamin was obtained 27% yield from the reaction of neopentane with vitamin B12r and O2 in the presence of excess V+3(aq) in 0.1 N HCl. This cobalamin was obtained in 27% yield. Isobutylcobalamin was isolated in 57% yield from...
isobutane at 2 atm of pressure. Both cobalamins were also synthesized by standard methods (reaction of the respective alkyl bromides with vitamin B\textsubscript{12a}).

**Discussion**

The present study demonstrates that alkanes and cycloalkanes can be converted into organic radicals under mild conditions and reacted with vitamin B\textsubscript{12r} to yield alkylcobalamins. The theoretical basis for this use of alkanes in reactions under O/R conditions is the same as in our previous studies [1, 2]. However, the fact that alkanes can be converted into alkylcobalamins is nevertheless surprising as alkanes are normally not used in synthetic organometallic chemistry. The new synthesis of alkylcobalamins is not of immediate practical use as alkylcobalamins are readily accessible by conventional methods of synthesis, e.g. by the reactions of alkyl halides with vitamin B\textsubscript{12a}. However, the functionalization of alkanes under O/R conditions is of interest because such reactions could occur in nature in certain hydrocarbon-metabolizing microorganisms. Since a corrin-dependent enzyme is involved in methane biosynthesis from C\textsubscript{1}-fragments, it would not be surprising if corrin-dependent enzymes were also utilized in methane assimilation or the metabolism of higher alkanes, although probably not in major pathways. Methane oxidizing bacteria are quite common in marine sedimentary materials and in soil, wherever methane and free oxygen are available [4]. Methane is oxidized typically to methanol, formaldehyde and formic acid, with cytochromes as electron transfer catalysts. A role of corrin dependent enzymes in hydrocarbon metabolism remains to be demonstrated.

**Experimental**

**Reagents and chemicals**

Vitamin B\textsubscript{12a} (hydroxocobalamin), was obtained from Merck, Sharp and Dohme Research Laboratories. Anhydrous VCl\textsubscript{3} was purchased from Organic/Inorganic Chemical Corp.; oxygen and the gaseous alkanes were purchased from Matheson as compressed gases, the liquid higher alkanes from Aldrich Chemical Corp., and D\textsubscript{2}O (99.8%) from Alfa-Ventron.

**Stock solutions**

A stock solution of V(III)aq. was prepared by dissolving 0.163 g of crystalline hydroxocobalamin in 20 ml of deaerated water. All stock solutions were stored in rubber-serum capped glass bottles under argon. For the deuterium exchange experiments, a V(III)-stock solution was prepared similarly as described above, in 1 M DCI.

**Methylcobalamin from methane**

A glass bottle of 38 ml capacity was fitted with a small magnetic stirrer, filled with CH\textsubscript{4} gas at 1 atm and closed with a rubber septum. Into this bottle, 0.5 ml of vitamin B\textsubscript{12} stock solution, 1.5 ml of 10\% NaOH, and 10 ml of O\textsubscript{2} gas (at 1 atm) were injected in this sequence. For best yields, more CH\textsubscript{4} was injected, e.g. enough to bring the pressure of CH\textsubscript{4} to 3 atm. After 2 h of reaction with stirring at room temperature, the bottle was opened and the cobalamins were phenol extracted. From the phenolic solution, the cobalamins were precipitated by the addition of acetone and ether. A sufficient amount of methylcobalamin was isolated by preparative thin layer chromatography on cellulose plates and identified by its absorption spectra before and after photolysis as well as by comparison with authentic methylcobalamin by chromatography on cellulose plates in 3 different solvent systems (see Table I).

**Methane/D\textsubscript{2}O exchange experiments**

A serum-capped glass bottle of 38 ml capacity was filled with CH\textsubscript{4} at 1 atm. Into this bottle, 2 ml of V(III) stock solution in 2 M DCI was added by means of a syringe, followed by 5 ml of O\textsubscript{2} gas at 1 atm, and 2 ml of 8 M NaOD. After 2 h of reaction at room temperature, gas samples were withdrawn for mass-spectrographic analysis. The presence of CH\textsubscript{3}D was established by the observed peak at m/e = 17. No above background CH\textsubscript{3}D was detectable in the gas samples from analogous experiments under strictly anaerobic conditions.

**Higher alkylcobalamins, branched alkylcobalamins and cycloalkylcobalamins**

The higher alkylcobalamins were prepared analogously to the synthesis of methylcobalamin described above. With liquid alkanes as the substrates, the reaction bottles were first filled with argon and the solutions of the reagents before 0.5 ml of the liquid hydrocarbons was injected by means of a syringe. With the hydrocarbons of chain length > 5, the reaction mixtures were ultrasonicated for 2 min. All organocobalamins were isolated. They were identified by thin-layer chromatography, spectroscopy and after photolysis, analysis of the gas-phase after photolysis, and comparison with authentic cobalamins synthesized by the reaction of vitamin B\textsubscript{12a} with the corresponding alkyl iodides or -bromides. Cycloalkycobalamins were prepared under acidic conditions by adjusting the initial pH to 1 or conducting the experiments in 0.1 M HCl. They were isolated by thin layer chromatography.
and identified spectroscopically before and after photolysis, analysis of the gas-phase hydrocarbons produced on photolysis, and comparison with authentic cycloalkylcobalamins. Neopentylcobalamin was prepared similarly using neopentane as the substrate at 2 atm of pressure. Isobutylcobalamin was synthesized in neutral solutions as described for methylcobalamin. For thin layer chromatography of these alkylcobalamins, solvent II of Table I was used, but 1 vol.% of glacial acetic acid was added (an acidic solvent mixture is required because of the instability of some of the more sterically hindered alkylcobalamins in neutral or alkaline solvent mixtures).