After the reaction with the phosphane the solvent was distilled off at $-30\,^{\circ}C$ under vacuum, the molecular organic compounds were extracted with CH_2Cl_2 at $-30\,^{\circ}C$, and the solid residue was dissolved in MeCN (main product: $[C_6F_5Xe][BF_4]$ (Table 1)). The extract of the reaction with the phosphane was characterized by ^{19}F NMR spectroscopy: $\delta = 1.32$ (d hept., $^1J(F,P) = 694.5$, $^4J(F,F) = 16.3$, 2F; PF_2), -132.73 (6F, *o*-F), -146.50 (3F, *p*-F), -159.48 (6F, *m*-F); GC-MS (70 eV): *m*/*z* (%): 570 (5) $[M^+]$, 551 (3) $[M^+ - F]$, 403 (100) $[M^+ - C_6F_5]$.

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Methylcorrinoids Methylate Radicals—Their Second Biological Mode of Action?**

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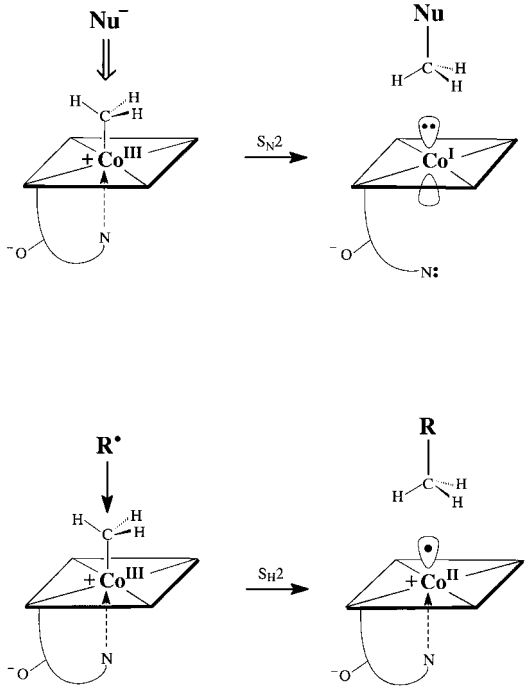
Dedicated to Professor Rolf Thauer on the occasion of his 60th birthday

The vitamin B_{12} derivative methylcobalamin (**1**), as well as related methylcorrinoids, are fundamentally important organometallic cofactors of methylation reactions.[1] The known enzymatic reactions with **1** depend on the heterolytic organometallic reactivities of methyl-Co^{III}-corrins and Co^{I}-corrins and the methylations proceed by nucleophilic substitution steps (Scheme 1, top).[2, 3] Extensive investigations of the B_{12}-dependent methionine synthase have indeed shown that the methyl transfer reactions occur by two nucleophilic substitution steps and convert homocysteine and N^5-methyltetrahydrofolate (with net retention) into methionine and tetrahydrofolate.[4]



Scheme 1.

Recent biosynthetic investigations on archaebacterial lipids[5a] and the antibiotics bottromycin,[5b] thienamycin,[6a] and thiostrepton[6b] provided evidence for unprecedented biological methylation reactions (and other alkylations) at saturated and inactivated carbon centers. In these methylation reactions methyl groups that originate from methionine are incorporated with net retention into the products.[5, 6] Arigoni and coworkers have considered a radical mechanism involving methylcorrin cofactors for these methylation reactions.[5a] Herein we report the first experiments that establish the (very efficient!) methylation of alkyl radicals by **1** (see Scheme 1, bottom).

Heating an oxygen-free aqueous solution of 2′-bis(ethoxycarbonyl)propylcobalamin (**2**)[7] and methylcobalamin (**1**)[1, 8] (**1**:**2** = 1.6:1) at 70 °C for about 5 h (at pH 7, with protection from light) completely decomposed the organocobalamin **2**. The organic decomposition products obtained after oxidation of the reaction mixture with air (in the dark and with the addition of potassium cyanide) were analyzed after extraction with deuterochloroform.[9] The 200 MHz ^1H NMR spectrum of the product exhibited signals for a 4.6:1 mixture of 2-ethyl-2-methylmalonic acid diethyl ester (**3**) and of 2,2-dimethylmalonic acid diethyl ester (**4**), with a combined yield amounting to approximately 70% (Scheme 2). In an analogous experiment using trideuteromethylcobalamin ([D_3]**1**) instead of **1** a combined yield of approximately 70% of [D_3]**3** and **4** was obtained as a 4.7:1 mixture (degree of deuteration of [D_3]**3**: 95 ± 5% from NMR studies). Storage of a deoxy-

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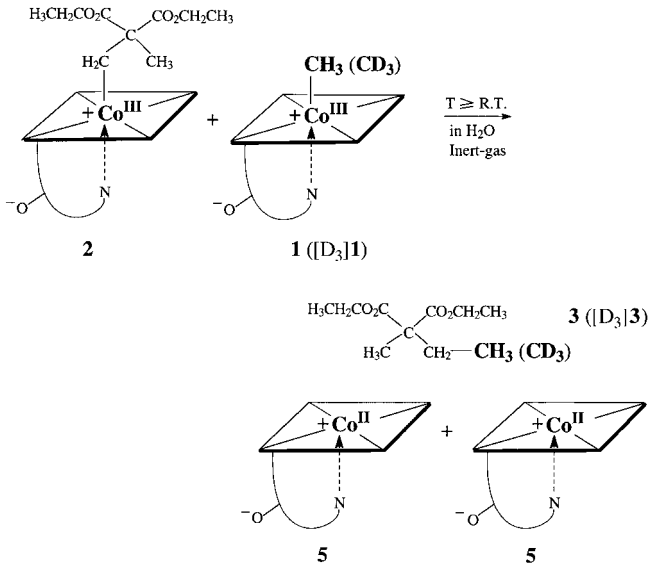


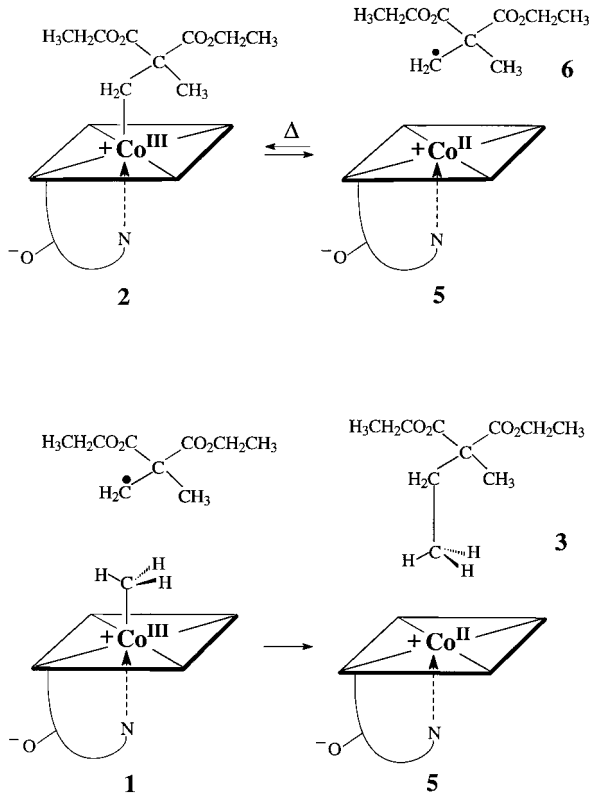
Scheme 2.

genated aqueous solution of **2** and **1** at room temperature and in the absence of light for about 17 h led to the decomposition of about 30% of **2** and to the formation of an approximately 3:1 mixture of **3** and **4**.

The organometallic vitamin B_{12} derivative **2** is thermally labile and decomposes even at room temperature to cob(II)alamin (**5**) and the 2-bisethoxycarbonylpropyl radical (**6**).[7, 10] The effective half-life of **2** at 27 °C in an oxygen-saturated, neutral aqueous solution is about 50 min, which is in qualitative agreement with earlier experiments.[7] However, **2** remains practically intact in a strictly oxygen-free aqueous solution (25 °C, pH 7), even after 20 h (UV/Vis). In a similar experiment **2** was completely decomposed after 7 h at 70 °C to Co^{II}-corrins (>90%, UV/Vis) and to 2,2-dimethylmalonic acid diethyl ester (ca. 40%).[11] In contrast, aqueous solutions of methylcobalamin (**1**) are stable for a practically unlimited time at room temperature (in the absence of nucleophiles and in the dark). In the presence of the radical trap 2,2,6,6,-tetramethyl-piperidinyl-1-oxyl (TEMPO) **1** is decomposed with an effective half-life of only about 4 h at 130 °C (in ethylene glycol).[12] Compound **1** thermolytically decomposes at 200 °C with formation of methane and ethane (ca. 1:1).[13]

Our experimental results can be explained by a decomposition of the thermolabile **2** in the presence of **1** to the Co^{II}-corrin **5** and to the 2-bisethoxycarbonylpropyl radical (**6**), which abstracts the cobalt-bound methyl group of **1**, to produce the methylation product **3** (Scheme 3).

The proposed abstraction of the cobalt-bound methyl group of methylcobalamin (**1**) by a primary alkyl radical (**6**) can be estimated from homolytic bond dissociation energies (BDE) to be a very exothermic reaction step ($\Delta H \approx -48$ kcal mol^{-1}).[14] Our experiments indicate that the abstraction of the oganometallically bound methyl group of **1** by the radical **6** competes effectively with the recombination reaction of **6** with the Co^{II}-corrin **5** (this recombination to **2** is also strongly exothermic and presumably nearly diffusion-controlled.[16]) We explain the formation of **3** from **1** and **2** by the substitution of the cobalt–corrin moiety of **1** by the alkyl radical **6**. Such



Scheme 3.

an intermolecular, homolytic substitution reaction by a (short-lived, organic) radical at a saturated carbon atom directly bound to a metal center is without precedence,[17] but corresponding reactions of (persistent) metal radicaloids (such as Co^{II}-complexes, etc.) are known.[18, 19] The occurrence of related intramolecular mechanisms was suggested to be responsible for the formation of (substituted) cycloalkanes from organometallic, bridged B_{12} dimers,[20] as well as from hexenyl cobaloximes.[18] Homolytic substitution reactions at carbon centers, when examined more closely, were found to take place with stereochemical inversion of configuration.[17, 18]

As a result of its characteristical low homolytic (Co-C)-BDE[12] **1** has the reactivity of a "latent" (persistent) methyl radical towards radicals[19, 21] and, similar to coenzyme B_{12} (**8**, a "reversibly functioning source of an alkyl radical"),[23] should be considered a partner in biological radical reactions. The enzymatic transfer of a methyl group from **1** to a carbon-centered radical should proceed with inversion of the configuration of the methyl group (and should therefore occur with net retention of configuration from methionine). In the known cases of unusual biosynthetic C-methylation reactions the configuration of the methyl group in the methylation product is the same as the one in methionine, the biosynthesis precursor.[5, 6] Our investigations thus support the suggested participation of methylcorrinoids in biosynthetic methylations of carbon radicals.[5]

The property of efficient radical methylation agent should, therefore, be added to the characteristic organometallic reactivities of methylcobalamin (**1**), the known biologically important methylation agent of nucleophiles.[2, 4] Methylcor-

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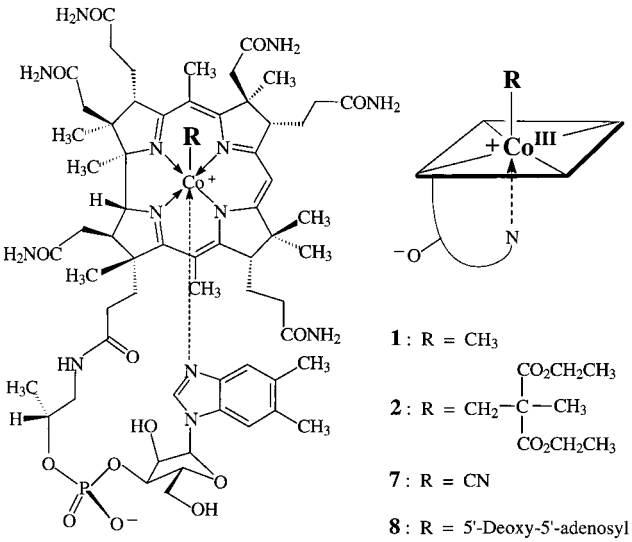

rinoids are to be considered also as "latent methyl radicals" and are cofactors suitable for homolytic biosynthetic methylation reactions.

Experimental Section

Trideuteromethylcob(III)alamin ([D_3]**1**): Prepared according to Tollinger et al.,[8] but using trideuteromethyl iodide instead of [^{13}C]methyl iodide. Degree of deuteration was determined as 98 ± 5% from ^{1}H NMR and FAB-MS studies.

2′-Bis(ethoxycarbonyl)propylcobalamin (**2**): An oxygen-free solution of aquocobalamin chloride (200 mg, 0.145 mmol) in aqueous 0.1M $LiClO_4$ (5 mL, < 10 ppm O_2) was reduced (UV/Vis) in a glove box at a mercury electrode to give cob(I)alamin (−1.1 V versus a 0.1N calomel reference (0.1 NCE), consumption: 2.06 F mol^{-1}). An 0.5M aqueous solution of *p*-toluene sulfonic acid (0.520 mL) and 2-bromomethyl-2-methylmalonic acid diethyl ester (0.178 mL)[25a] (0.867 mmol) in methanol (1 mL) were then added in the dark. The electrolysis was continued at −1.0 V versus 0.1 NCE for 2.5 h. The solvents were removed at room temperature. The residue was dissolved in a little methanol and precipitated with acetone. The precipitate was washed with acetone and dried to give **2**-**H**$^+$ (as the tosylate, 231 mg, 94% yield) as an orange coloured solid (still containing 5% aquocobalamin). UV/Vis (H_2O, pH 2): λ_{max} (l g ε) = 451 s (3.76), 421 (3.78), 296 s (4.17), 285 (4.23), 266 (4.30); ^{1}H NMR (500 MHz, D_2O, pD 2): δ = 9.10 (s, 1H), 7.55 (d, 2H), 7.41 (s, 1H), 7.35 (s, 1H), 7.22 (d, 2H), 7.04 (s, 1H), 6.40 (d, 1H), 4.82 (dd, 1H), 4.66 (dd, 1H), 4.62 (m, 1H), 4.58 (d, 1H), 4.21–4.15 (m, 1H), 4.07 (d, 1H), 3.92–3.85 (m, 2H), 3.80–3.72 (m, 4H), 3.64 (dd, 1H), 3.43 (d, 1H), 3.18 (dd, 1H), 3.06 (dd, 1H), 2.77–2.64 (m, 3H), 2.45–2.08 (m, 10H), 2.27 (s, 6H), 2.24 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.04–1.89 (m, 5H), 1.80–1.60 (m, 4H), 1.75 (s, 3H), 1.53 (s, 3H), 1.40 (s, 3H), 1.30 (s, 3H), 1.29 (d, 1H), 1.15 (s, 3H), 1.03 (d, 3H), 0.95 (t, 3H), 0.86 (t, 3H), 0.54 (d, 1H), 0.50 (s, 3H), −0.07 (s, 3H); FAB-MS (*o*-nitrobenzyl alcohol (NOBA) matrix): *m*/*z* (%): 1518.5/1517.5/1516.5 (17/22/28) [*M*+H$^+$]; 1331.5/1330.5/1329.4 (38/76/100) [*M*+H$^+$ − $C_9H_{15}O_4$].

Thermolysis of **2** in presence of methylcobalamin (**1**): **2**-**H**$^+$ (30.0 mg containing 17% water, 0.0135 mmol) was dissolved in hydrochloric acid (3 mL, 10 mM). The solvents were removed and the solid residue was dried. Similarly **1** (29.0 mg, 0.0216 mmol) was dissolved in water (5 mL) and then dried. Both compounds were then dissolved in phosphate buffer (4 mL, 0.1M, pH 7) in the dark and in the glove box, and the deoxygenated mixture was heated in the dark at 70 °C for 5 h. Oxygen-saturated water (2 mL) and 0.5M aqueous KCN (0.054 mL) were then added and the mixture was extracted with $CDCl_3$ (3 × 0.6 mL). The extract was dried by passing through a plug of dried cotton wool. Analysis by 200 MHz ^{1}H NMR spectroscopy identified the product as a 4.6:1 mixture of 2-ethyl-2-methylmalonic acid diethyl ester (**3**)[25b] and 2,2-dimethyl-malonic acid diethyl ester (**4**).[25c] The total yield (ca. 70%) was estimated by comparison with the signal of the residual $CHCl_3$ in $CDCl_3$ (standardized against anthracene). **3**: ^{1}H NMR (200 MHz, $CDCl_3$):[25b] δ = 4.19 (q, *J* = 7.1, OEt), 1.92 (q, *J* = 7.5, Et-C(2)), 1.39 (s, CH_3-C(2)), 1.25 (t, *J* = 7.1, OEt), 0.88 (t, *J* = 7.5, Et-C(2)).

Thermolysis of **2** and trideuteromethylcobalamin ([D_3]**1**): The reaction was carried out as above, but with [D_3]**1** instead of **1**, and gave 2-(2,2,2-trideuteroethyl)-2-methylmalonic acid diethyl ester ([D_3]**3**) and **4** (4.7:1, 71%). [D_3]**3**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.19 (q, *J* = 7.1, OEt), 1.89 (br s, Et-C(2)), 1.39(s, CH_3-C(2)), 1.25(t, *J* = 7.1, OEt).

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[9] The aqueous fraction of a similar experiment contained vitamin B_{12} (**7**) and **1** in the ratio 1.7:1.0 (^{1}H NMR).

[10] In acidic solutions **2** is protonated at the nucleotide base to give **2**-**H**$^+$, which tends to decompose more than 100 times less rapidly than **2**.

[11] The NMR data provided no evidence for the existence of products derived from a rearrangement of **6** to a methylsuccinyl radical (for studies with **6** and with related radicals in solution, see S. Wollowitz, J. Halpern, *J. Am. Chem. Soc.* **1988**, *110*, 3112–3120).

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[21] The observation of ethane as the main product of the photolysis[13] and of the reductive electrolysis[22] of **1** was rationalized by dimerization of methyl radicals.[13, 22] Our results suggest an alternative consideration, namely of a direct reaction of methyl radicals with the intact methylcorrinoid (**1**).

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