(17) This program was written by J. A. Ibers.
(18) (a) $[Cp_4Fe_4S_4]^z$: z = 0, R. A. Schunn, C. J. Fritchie, Jr., and C. T. Prewitt, *Inorg. Chem.*, **5**, 892 (1966); C.-H. Wei, G. R. Wilkes, P. M. Treichel, and L. F. Dahl, *ibid.*, **5**, 900 (1966). z = 1+, Trinh-Toan, W. P. Fehlhammer, and L. F. Dahl, *J. Am. Chem. Soc.*, **99**, 402 (1977). z = 2+, Trinh-Toan, B. K. Teo, J. A. Ferguson, T. J. Meyer, and L. F. Dahl, *ibid.*, **99**, 408 (1977). (b) $Fe_4S_4(NO)_4$: R. S. Gall, C. T.-W. Chu, and L. F. Dahl, *ibid.*, **96**, 4019 (1974). (c) $[Fe_4S_4(S_2C_2(CF_3)_2)_4]^{2-}$: I. Bernal, B. R. Davis, M. L. Good, and S. Chandra, *J. Coord. Chem.*, **2**, 61 (1972). (d) $[Re_4S_4(CN)_{12}]^{4-}$: M. Laing, P. M. Kiernan, and W. P. Griffith, *J. Chem. Soc., Chem. Commun.*, 221 (1977).
(19) For high-spin $Fe^{II,III}$–SR distances cf. (a) R. W. Lane, J. A. Ibers, R. B. Frankel, G. C. Papaefthymiou, and R. H. Holm, *J. Am. Chem. Soc.*, **99**, 84 (1977); (b) D. Coucouvanis, D. Swenson, N. C. Baenziger, D. G. Holah, A. Kostikas, A. Simopoulos, and V. Petrouleas, *ibid.*, **98**, 5721 (1976).
(20) C. W. Carter, Jr., *J. Biol. Chem.*, **252**, 7802 (1977).
(21) For the four maximally congruent orientations the following deviations in distances between proximal vertices of the two core polyhedra were found: Fe···Fe, 0.028, 0.035, 0.009, 0.023 Å (mean 0.024 Å); S*···S*, 0.048, 0.032, 0.057, 0.032 Å (mean 0.042 Å).
(22) The display in Figure 1 of maximally congruent dianion–trianion core orientations precludes presentation of maximally congruent trianion–trianion orientations without recourse to additional structural depictions (not shown).
(23) For these orientations the following information corresponding to that in footnote 21 was obtained: Fe···Fe, 0.044, 0.036, 0.050, 0.022 Å (mean 0.038 Å); S*···S*, 0.057, 0.024, 0.042, 0.034 Å (mean 0.039 Å).
(24) To ensure that there were no systematic errors in the methods applied to the solution and refinement of the structure reported herein, a second data set was collected on a second crystal of similar size using an Enraf-Nonius CAD4 diffractometer and Mo $K\bar{\alpha}$ radiation. The data were processed as described in the Experimental Section and the structure was refined to convergence using the parameters reported in Table III as starting values. A comparison of the final set of coordinates with the data of Table III revealed no deviations greater than about 3σ for the anions, with deviations of the core atom coordinates averaging less than 1σ. A comparison of the 12 Fe–S bond distances of the core refined from the two different data sets revealed a mean difference of 0.003 Å (while the average error in a single Fe–S bond distance as estimated from the variance-covariance of the least squares was 0.005 Å). The greatest difference in Fe–S distances observed was 0.008 Å. To further ensure that there were no significant systematic errors in the refinement procedure, a second refinement was carried out on the CAD4 data set using the NUCLS program of Ibers. In this refinement the phenyl ring carbon atoms of the anions were treated as rigid groups with each atom in the group having its own isotropic temperature factor. The methylene carbons of the benzyl groups were also refined anisotropically. Inspection of the positions for the 12 core atoms from each of the refinements of this second data set revealed an average difference in fractional cell coordinates of 0.000 31 with least-squares estimated errors of 0.0003. In terms of distances, the average difference for the 12 Fe–S bonds was 0.0038 Å with the average estimated error of an individual distance being 0.005 Å. The largest difference observed was 0.011 Å. The two data sets, refined to give three separate sets of coordinates, all resulted in essentially the same core structure for the $[Fe_4S_4(SCH_2Ph)_4]^{3-}$ anion. The greatest difference among the core atomic coordinates of any of the three sets was 3.4σ and the greatest difference in any Fe–S bond distance was 0.019 Å. The average coordinate and Fe–S distance differences between any set were about 1σ. These results conclusively eliminate systematic errors in data collection, processing, and refinement as being responsible for the observed nontetragonal core distortion for the structure of $[Fe_4S_4(SCH_2Ph)_4]^{3-}$.
(25) This process is described more fully elsewhere.[7]
(26) E. J. Laskowski, J. G. Reynolds, R. B. Frankel, S. Foner, G. C. Papaefthymiou, and R. H. Holm, results to be published.
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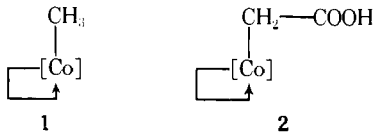
Studies on Vitamin B_{12} and Related Compounds. 49. Direct Synthesis of Alkyl- and of ω-Carboxyalkylcobalamins from Vitamin B_{12} and Aliphatic Carboxylic Acids under "Oxidizing–Reducing" Conditions[1]

Gerhard N. Schrauzer* and Masao Hashimoto[2]

Contribution from the Department of Chemistry, University of California at San Diego, Revelle College, La Jolla, California 92093. *Received September 22, 1978*

Abstract: Straight-chain aliphatic carboxylic acids $C_nH_{2n+1}COOH$ (n =, e.g., 1-6) and certain dicarboxylic as well as branched carboxylic acids can be oxidized to organic radicals in the presence of vitamin B_{12r} and converted into organocobalamins. Owing to the instability of secondary and tertiary alkylcobalamins and other steric effects, vitamin B_{12r} acts as a selective scavenger of primary alkyl and ω-carboxyalkyl radicals. Accordingly, *n*-alkylcobalamins and ω-carboxyalkylcobalamins are isolated exclusively and often in quantitative yields. The alkyl and carboxyalkyl radicals are generated from the carboxylic acids by $HO_2\cdot$, $O_2^{-}\cdot$, or HO· radicals formed in reactions of limiting amounts of O_2 preferably with V(III) salts, although V(IV), Mo(III), and certain other reducing metal ions can also be used. Oxygen can furthermore be replaced by H_2O_2, by Fenton reagent, or by electrochemically generated oxygen or oxygen radicals. The reducing metal ions, e.g., V(III), must be present in excess. They not only act as activators of oxygen, but also as reductants of vitamin B_{12}, thus maintaining it in the reactive Co(II) state. The new method of synthesis of organocobalamins under "oxidizing-reducing" conditions may become useful for the preparation of otherwise difficultly accessible compounds.

The starting point of the present study was the observation that *methylcobalamin* (**1**) and *carboxymethylcobalamin* (**2**)[3]



1 2

were formed in a reaction in which acetate-buffered solutions of vitamin B_{12r} were reacted with limiting amounts of oxygen in the presence of V^{3+}(aq) ions.

The question of how methylcobalamin could be synthesized from acetic acid and vitamin B_{12} under nonenzymatic conditions has intrigued the senior author for many years, following the report that this reaction occurs, either directly or indirectly, in cells or cell extracts of the methanogenic bacterium *Methanosarcina barkeri*.[4] Assuming that this reaction occurs directly, many unsuccessful attempts were made in our laboratory to verify this reaction under nonenzymatic conditions, suggesting that a specific activation of acetate was necessary or that the transfer occurred indirectly. The new observation indicated that **1** can be formed from acetate by way of an ox-

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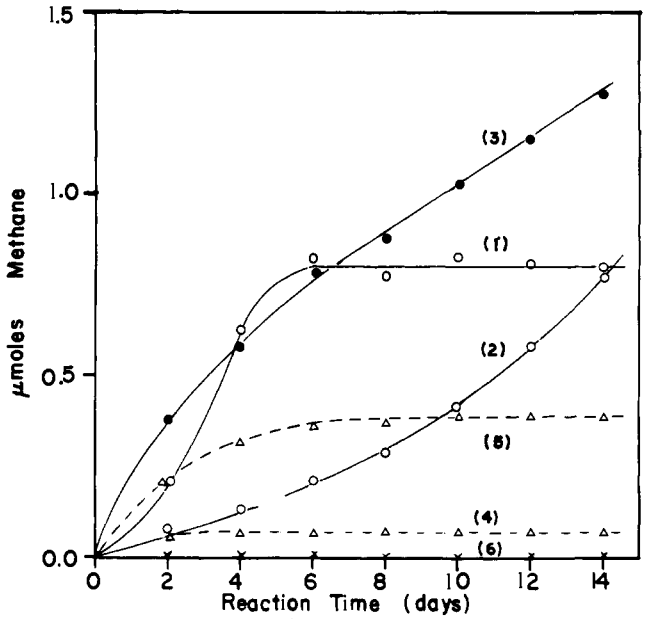


Figure 1. Time-yield plots of methane production from acetate buffered aqueous solutions of V(III), V(IV), and V(V) at 60 °C. Reaction solutions contained, in a total solution volume of 10 mL, acetate (10 mmol) (initial pH adjusted with 10% NaOH), V(III, IV, V) (0.2 mmol), and oxygen concentrations as follows: (1) —○—, O_2 (initial), 145 μmol, pH 4.5 [V^{+3}(aq)]; (2) —○—, O_2 (initial), 40 μmol, pH 4.5 [V^{+3}(aq)]; (3) —●—, O_2 (initial), 40 μmol, pH 1.6 [V^{+3}(aq)]; (4) - - -△- -, O_2 (initial), 135 μmol, pH 5.0 [VO^{+2}(aq)]; (5) - - -▲- -, O_2 (initial), 35 μmol, pH 5.0 [VO^{+2}(aq)]; (6) —x—, O_2 (initial), 40 μmol, pH 4.5 [VO_2^+ (aq)].

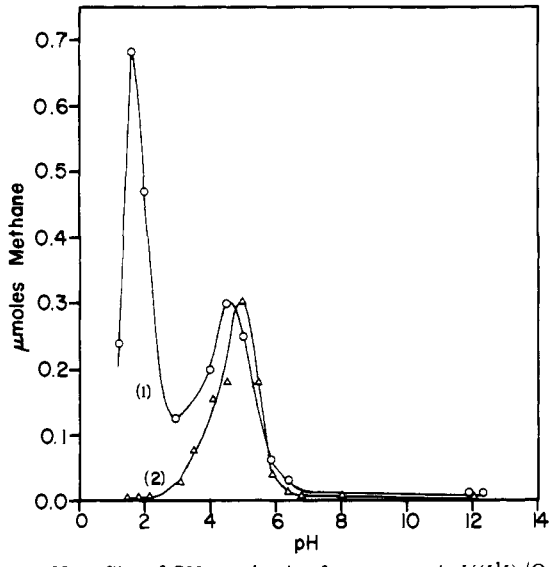


Figure 2. pH profiles of CH_4 production from acetate in V(III)/O_2 (—○—○—) and V(IV)/O_2 (—△—△—) systems. Reaction solutions contained, in a total solution volume of 10 mL, CH_3COOH, pH adjusted with 10% aqueous NaOH; V^{3+}(aq), VO^{2+}(aq), (initial concentrations) 0.2 mmol; O_2 (initial), 35 μmol in argon atmosphere. Methane yields were measured after 5 days of reaction at 60 °C.

$$CH_3COOH \xrightarrow[-e^-,-H^+]{} CH_3COO\cdot \xrightarrow[-CO_2]{} CH_3\cdot \xrightarrow{+[Co^{2+}]} CH_3\text{—}[Co] \quad (1)$$

idative decarboxylation in terms of reaction eq 1. Even though the enzymatic reaction occurs under anaerobic conditions whereas eq 1 requires the presence of traces of oxygen, the use of vitamin B_{12r} as a scavenger of free radicals appeared to offer interesting synthetic prospects and was investigated further.

A reaction related to eq 1 which occurs with the abstraction of a hydrogen atom from the methyl group of acetate could be responsible for the formation of **2** (eq 2). The oxidative de-

$$CH_3COO^- \xrightarrow[-H]{} \cdot CH_2COO^- \xrightarrow{+[Co^{2+}],\ H^+} CH_2(COOH)\text{—}[Co] \quad (2)$$

carboxylation of aliphatic carboxylic acid by oxygen radicals generated by activated oxygen or Fenton reagent is well-known, as is the abstraction of aliphatic hydrogen atoms from acetic acid.[5] We have verified eq 2 both with O_2 and with Fenton reagent as the oxidants in the presence of V(III) salts and vitamin B_{12a} (hydroxocobalamin), which is reduced under these conditions to vitamin B_{12r}. To prevent its reoxidation during the reaction it is necessary to operate at low concentrations of oxidant in the presence of a large excess of V(III). The high yields of **1** and **2** obtained prompted us to extend our studies to a number of higher carboxylic acids and some dicarboxylic acids as the substrates. In this manner practical methods of synthesis of organocobalamins under seemingly contradictory conditions have become available. For comparative purposes, some of the oxidation-reduction reactions of the carboxylic acids were also performed in the absence of added vitamin B_{12}. These will be described first.

Results

Oxidative Degradation of Carboxylic Acids by Oxygen in the Presence of Vanadium Ions. Aqueous acetate does not appear to undergo any unusual chemical reactions with solutions of V(III) or V(IV) ions, even after prolonged heating to 60 °C, if oxygen is rigorously excluded. Upon the admission of traces of air, a slow evolution of CH_4 with concomitant production of CO_2 is observed on standing at room temperature and more rapidly on heating to 60 °C. Upon further injection of limiting amounts of oxygen, a continuous evolution of CH_4 from acetate can be sustained for days and weeks until all the vanadium is oxidized to the pentavalent state. Aqueous solutions of vanadyl (IV) sulfate also induced the oxidative breakdown of acetate, but in this case the reactions ceased earlier owing to the more rapid irreversible oxidation of V(IV) to V(V). Examples of time-yield plots from several experiments are shown in Figure 1. The formation of CO_2 was demonstrated qualitatively, but was not followed quantitatively (see Experimental Section). In addition to CH_4, traces of C_2H_6, always less than 1% of the amount of CH_4, and variable amounts of CH_3Cl were detected in the gas phase. Analysis of the reaction solutions revealed the absence of alcohols, aldehydes, or succinic acid. The latter could have formed through the coupling of two $\cdot CH_2COOH$ radicals, but this reaction does not occur under our reducing reaction conditions.

Figure 2 shows the pH profiles for the formation of CH_4 from acetate in V(III)/O_2 and V(IV)/O_2 systems.

The evolution of CH_4 could not be measurably influenced through the simultaneous exposure of the reaction solutions to visible or near-UV light. On heating, the rates of CH_4 production demonstrated an increase in the temperature range between 20 and 120 °C. With five data points, linear Arrhenius plots were obtained from which the apparent energy of activation was calculated to be 9.54 ± 0.05 kcal/mol. Carbon monoxide exhibited no inhibitory effects on CH_4 production, but complexing agents such as catechol, salicylaldehyde, phthalic acid, and EDTA caused a significant diminution. The addition of methanol and of other aliphatic alcohols also resulted in inhibitory effects on CH_4 production, apparently because they are oxidized themselves. The oxidative degradation of CH_3COOH in D_2O with V(III)/O_2 at pH 4.5 proceeded at the same rate as the corresponding reaction in H_2O, affording mainly CH_3D, as evidenced from mass-spectro-

Table I. Hydrocarbons in the Gas Phase from Reactions of Carboxylic Acids Initiated with O_2 in the Presence of V(III)[a]

acid	hydrocarbon products in the gas phase	ratios
CH_3COOH	CH_4 (C_2H_6)	>99/<1
C_2H_5COOH	C_2H_6, CH_4	100:6
n-C_3H_7COOH	C_3H_8, C_3H_6, C_2H_6, CH_4	100:86:13:11
i-C_3H_7COOH	C_3H_8, C_3H_6, CH_4	100:6:17
n-C_4H_9COOH	C_4H_{10}, C_3H_8, C_2H_6, CH_4	22:19:100:8
n-$C_5H_{11}COOH$	C_5H_{12}, C_4H_{10}, C_3H_8, C_2H_6, C_2H_4, CH_4	100:13:46:53:tr:20
n-$C_6H_{13}COOH$	C_6H_{14}, C_5H_{12}, C_4H_{10}, C_3H_8, C_2H_4, CH_4	100:35:8:23:65:37:31
$CH_2(COOH)_2$	CH_4, CH_3COOH	
$HOOCCH_2CH_2COOH$	C_2H_4, (CH_4), C_2H_5COOH	

[a] Reaction conditions: 10 mmol each of the carboxylic acids was added into glass bottles of 38-mL capacity and 10 mL of 10% NaOH was added to adjust the solution pH to 4.5. The solutions were diluted to a volume of 10 mL by addition of H_2O and the bottles sealed with silicone rubber septa. After the air in the bottles was displaced with argon, 0.5 mL of the VCl_3 stock solution (=0.2 mmol, see Experimental Section) was injected, followed by 4.4 mL of air at 1 atm of pressure (40 μmol of O_2). The bottles were then heated at 60 °C for 5 days before samples of the gas phase were withdrawn for product analysis by GLC.

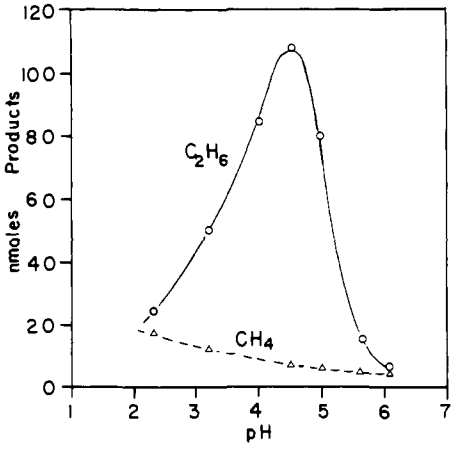


Figure 3. pH profile of hydrocarbon production from C_2H_5COOH. Reaction conditions are as outlined in legend to Table I except for differences in initial pH. Yields of hydrocarbons in the gas phase were determined after 5 days of reaction at 60 °C.

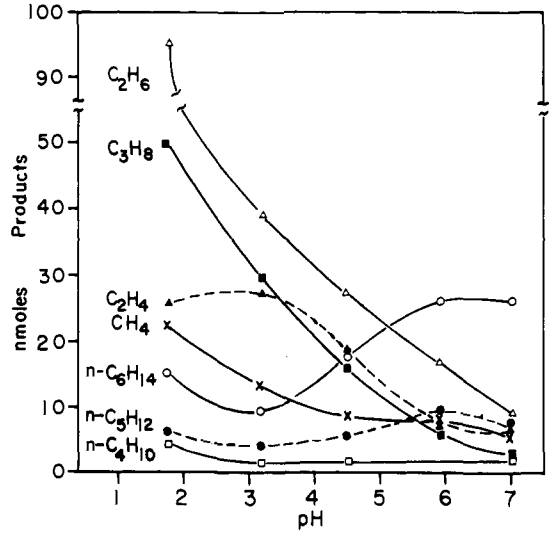


Figure 4. pH profile of hydrocarbon production from n-$C_6H_{13}COOH$ under the reaction conditions given in the legend of Table I, except that yields were measured after 7 days of reaction and experiments were run at different pH values.

graphic analysis. Exposure of higher saturated linear carboxylic acids to V(III)/O_2 produced results similar to those observed with acetic acid as the substrate. Hydrocarbons evolved slowly at room temperature but more rapidly on heating to 60 °C. However, the hydrocarbons in the gas phase consisted of mixtures which indicated extensive oxidative destruction of the substrates (see Table I), while free-radical coupling products were as a rule absent. Only in the case of acetic acid, traces of C_2H_6 were observed in addition to CH_4. The higher carboxylic acids gave rise to mixtures of saturated and unsaturated hydrocarbons which increase in complexity with increasing chain length of the paraffinic residue. The rates of hydrocarbon evolution are pH dependent. For propionate as the substrate, a pH profile is shown in Figure 3. For heptanoic acid (n-$C_6H_{13}COOH$), the pH profile is reproduced in Figure 4. As with acetic acid, the evolution of hydrocarbons from the reaction solutions is slow but continues for several days and until the oxidation of V(III) to V(V) is complete. The latter reaction is dependent on the initial amounts of O_2 present, but this dependence was not followed for acids other than acetic (see Figure 1).

The oxidative decarboxylation of acetate could also be initiated by metal/oxygen couples other than V(III)/O_2, e.g., with Mo(III)/O_2, Pd(II)/O_2, Fe(II)/O_2, and Cu(I)/O_2, but the vanadium/O_2 systems were the most active.

The evolution of CH_4 from acetate in reactions initiated by V(III)/O_2 is inhibited by Cu^{2+}, which causes increased amounts of CH_3Cl to be formed instead. This is attributed to the oxidation of CH_3· radicals, as is well-known.[6]

Reduction of Vitamin B_{12a} with Vanadium(III) and -(IV). Neutral and acidic solutions of V(III) salts reduce vitamin B_{12a} to vitamin B_{12r} (see Figure 5). Suspensions of $V(OH)_3$ in aqueous NaOH reduce vitamin B_{12a} even to vitamin B_{12s}. On reaction with air, vitamin B_{12s} is rapidly oxidized, resulting in a brown solution containing vitamin B_{12r}. Hence, reactions of vitamin B_{12r} can also be studied under conditions favoring the formation of vitamin B_{12s}, provided that appropriate amounts of oxygen are admitted to the system.

The reducing properties of VO^{2+} are insufficient to cause a reduction of vitamin B_{12a} to vitamin B_{12s}, but neutral or weakly acidic solutions of VO_3^{2-}·aq reduce vitamin B_{12a} cleanly to vitamin B_{12r}, especially on heating to about 60 °C. Spectrophotometric traces of this reduction are similar to those in Figure 5 but are not shown.

Oxidative Degradation of Acetic Acid in the Presence of Vitamin B_{12r}. The formation of organocobalamins from acetic acid and vitamin B_{12r} was first suspected in experiments in which vitamin B_{12a} was reduced to vitamin B_{12r} with V(III) salts in acetate buffer. Under most rigorously anaerobic conditions, the reduction of the vitamin to the Co(II) state proceeded normally, but in the presence of traces of air evidence for the formation of some light-sensitive organocobalamins was obtained. In addition, some methane was detected in the gas phase, but only if the reactions were run in normal daylight. The evolution of CH_4 was minimal when the experiments were conducted in the dark, but on subsequent exposure of the re-

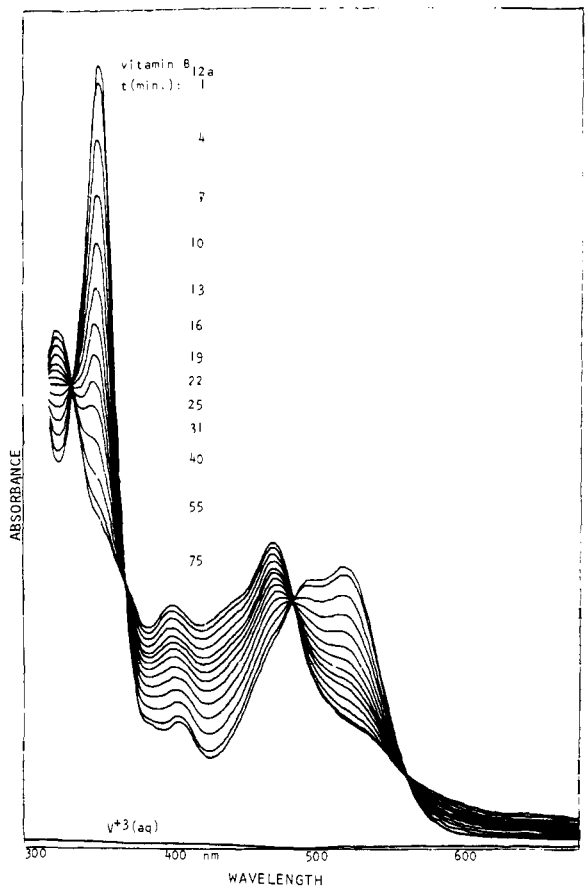


Figure 5. Reduction of hydroxocobalamin with V^{3+}(aq) at pH 4.5 with V^{3+}(aq). Reaction solutions contained, in a total volume of 2.5 mL of 1 M acetate buffer, vitamin B_{12a} (0.1 μmol) and V^{3+}(aq) (0.2 μmol) (initial concentrations), under argon at 25 °C.

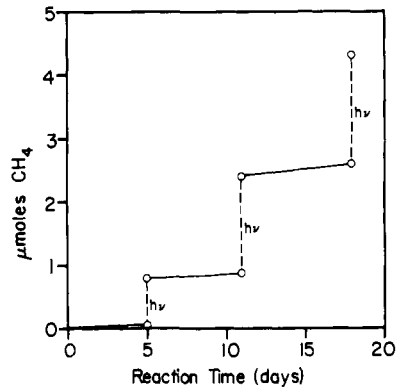


Figure 6. Methane evolution from pH 4.5 buffered solutions of acetate in the presence of vitamin B_{12r}, V^{3+}(aq), and limiting amounts of O_2. Reaction solutions contained, in a total volume of 10 mL, CH_3COOH (10 mmol), vitamin B_{12r} (7 μmol), and V^{+3}(aq) (0.2 mmol). The initial concentration of O_2 was 35 μmol in the argon atmosphere. The reaction temperature was 60 °C. At specified time points, reaction solutions were irradiated with a 150-W tungsten filament projector-spot (GE) lamp.

action solutions to light significant amounts of CH_4 as well as traces of C_2H_6 were generated. This phenomenon was repeatable (see Figure 6). Workup of the unphotolyzed reaction solutions by phenol extraction and analysis of the isolated corrins by thin layer chromatography (TLC) revealed the presence of **1** and of a second organocobalamin later identified as **2**. The identity of the two compounds was confirmed by comparison with alternatively synthesized samples; **1** was prepared from CH_3I and vitamin B_{12s}, **2** by the corresponding reaction of B_{12s} with $BrCH_2COOH$; the compounds were purified as described elsewhere.[7,8,9a]

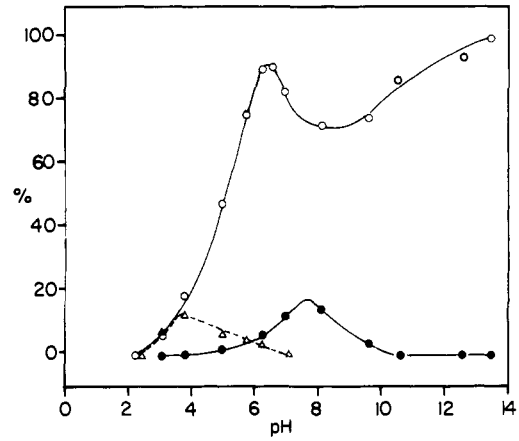


Figure 7. Formation of carboxymethylcobalamin (—O—O—), of methylcobalamin, (- - -Δ- - - -Δ- - -), and of yellow oxidized corrin (—●—●—) from acetate, V(III), and air (excess) at room temperature after 1 day of reaction. Yields are in percent based on total vitamin B_{12} employed. The formation of side-chain-hydrolysis products of carboxymethylcobalamin, which becomes noticeable above the pH of 13, is not indicated. At t = 0, the reaction solutions contained 2.5 μmol of vitamin B_{12r} and 10 mmol of CH_3COOH (pH adjusted with 10% aqueous NaOH) in a total solution volume of 10 mL. The air volume in contact with the reaction solutions was 28 mL at 25 °C, the air pressure, 1 atm.

Methylcobalamin (**1**) is formed preferentially in solutions of pH <4.5. Carboxymethylcobalamin (**2**) is formed in virtually quantitative yields in the pH range of 5–7. It is also still the main product if the reactions are conducted between pH 7 and 10, but under these conditions the formation of yellow oxidized corrins[9b] becomes a disturbing side reaction, consuming up to 10% of the total cobalamin. Above pH 11, at least four new cobalamins are formed as evidenced by TLC. These were identified as derivatives of **2** with hydrolyzed propionamide side chains (the same products were generated by treating **2** with dilute NaOH). These compounds exhibit optical absorption spectra identical with those of **2** and the usual light sensitivity of organocobalamins. Figure 7 shows the pH dependence of the formation of all cobalamin products; Table II lists their R_f and $R_{B_{12}}$ values in two different solvent systems. The reaction of acetate with limiting amounts of oxygen in the presence of V(III) and of vitamin B_{12r} was also carried out on a preparative scale, affording **2** in 95% yield (see Experimental Section).

The oxidative degradation of acetate can also be initiated with H_2O_2 in place of oxygen. In the presence of V(III) salts at pH 4.5, a 7:1 mixture of CH_4 and C_2H_6 was formed, indicating that the oxidative decarboxylation had occurred. The same experiment in the presence of added vitamin B_{12a} afforded **1** and **2** in addition to CH_4 and C_2H_6. The two organocobalamins were also detected if the oxidation was performed with Fenton's reagent in the presence of vitamin B_{12a}, V(III) salt, and acetate. To obtain the two organocobalamins in high yields with minimal formation of yellow corrin oxidation product(s), H_2O_2 must be added slowly and in dilute solution to avoid local excesses of oxidant. On a micromolar scale and a pH of about 1, methylcobalamin was obtained from vitamin B_{12r}, acetic acid, and $Fe^{2+}/H_2O_2/V(III)$ in 75.9% yield. Under these conditions, 9.1% of the total corrin was converted to **2**, and 15% was recovered as vitamin B_{12a}. The same experiment at pH 4.25 afforded 70.2% of **1** and 22.8% of **2**, with 7.0% of unreacted vitamin B_{12} remaining. No yellow corrin oxidation products formed under these conditions (see Experimental Section).

Carboxymethylcobalamin was also formed in almost quantitative yields if the oxidation of acetate was initiated with electrochemically generated oxygen or oxygen radicals. A

Table II. Characterization of Cobalamin Reaction Products from the Induced Oxidation of Acetate in the Presence of Vitamin B_{12r} by Thin Layer Chromatography

no.	compd	solvent system I[a]		solvent system II[b]	
		R_f	$R_{B_{12}}$	R_f	$R_{B_{12}}$
1	methylcobalamin[c]	0.47	2.66	0.47	6.82
2	carboxymethylcobalamin[c]	0.37	2.07	0.20	2.87
3	hydrolysis products of **2**	0.45	2.51	0.01	0.2[d]
		0.48	2.70	0.07	1.0[d]
		0.55	3.07	0.10	1.4[d]
		0.62	3.47	0.11	1.6[d]
4	hydroxocobalamin	0.18	1.00	0.07	1.00

[a] H_2O/saturated *sec*-butyl alcohol, 1 vol % CH_3COOH. [b] H_2O/saturated *sec*-butyl alcohol, 1 vol % NH_4OH. $R_{B_{12}} = R_f$ value relative to vitamin B_{12a}. [c] R_f and $R_{B_{12}}$ values are identical with those of authentic compounds. [d] Not well resolved.

Table III. Alkylcobalamins and Carboxyalkylcobalamins Prepared from Carboxylic Acids by Oxidative Degradation in the Presence of Vitamin B_{12r}

acid	*n*-alkylcobalamins			ω-carboxyalkylcobalamins		
	R	method[a]	R_f[b]	R	method[a]	R_f[b]
CH_3COOH	$-CH_3$	A, ***B*** (76)	0.45	$-CH_2COOH$	***C, D*** (100)	0.34
$CH_2(COOH)_2$				$-CH_2COOH$	A, ***B*** (100)	0.34
C_2H_5COOH	$-C_2H_5$	A, ***B*** (15)	0.46	$-CH_2CH_2COOH$	***C, D*** (100)	0.35
$(-CH_2COOH)_2$				$-CH_2CH_2COOH$	A, ***B*** (99)	0.35
n-C_3H_7COOH	$-C_3H_7$	A, ***B*** (29)	0.50	$-(CH_2)_3COOH$	***C, D*** (100)	0.41
i-C_3H_7COOH				$CH_3CH(CH_2)COOH$	***B, C, D*** (100)	0.40
n-C_4H_9COOH	$-C_4H_9$	A, ***B*** (3)	0.53	$-(CH_2)_4COOH$	B, C, ***D*** (87)	0.44
n-$C_5H_{11}COOH$	$-C_5H_{11}$	A, ***B*** (1)	0.57	$-(CH_2)_5COOH$	C, ***D*** (78)	0.47
$(CH_3)_3CCOOH$				$-CH_2C(CH_3)_2COOH$	A, ***B***, C, (60)	0.47
n-$C_6H_{13}COOH$	$-C_6H_{13}$	B (1)	0.60	$-(CH_2)_6COOH$	C, ***D*** (57)	0.51

[a] Methods of synthesis: A, V(III)/O_2(acidic); B, V(III)/H_2O_2-Fe^{2+} (acidic); C, V(III)/O_2 (alkaline); D, V(III)/electrolysis (alkaline). Preferred method italicized; yields, based on total cobalamin, in parentheses. [b] Solvent system: water/saturated 2-butanol + 1 vol % CH_3COOH. TLC performed on cellulose plates (R_f for vitamin B_{12a} 0.15).

simple electrolysis cell with Pt electrodes was used. At a voltage of 4.5 V and the current of 60 mA at room temperature, the reaction was complete after 7 min of electrolysis.

Oxidative Degradation of Higher Carboxylic Acids in the Presence of Vitamin B_{12r}. The reaction of higher carboxylic acids with vitamin B_{12r} in the presence of V(III) or V(IV) plus limiting amounts of oxygen, or by any of the alternative methods of oxidation outlined above, gives rise to the formation of isolable alkyl- and carboxyalkylcobalamins. These were purified by phenol extraction and identified as *n*-alkyl- and ω-carboxyalkylcobalamins. For synthetic purposes, the use of Fenton reagent in place of O_2 is recommended. With H_2O_2/V(III) mixtures alone the yields of organocobalamins are usually lower than with Fenton reagent. Hence, this method of synthesis was not explored further. For the preparation of the ω-carboxyalkylcobalamins, both the electrochemical method as well as air oxidation of alkaline reaction solutions produced satisfactory yields, particularly with the lower carboxylic acids as the substrates. The yields of organocobalamins generally were found to decline with increasing chain length of the acid chosen; this is in part due to diminished solubility. We have been unable thus far to obtain a ω-stearylcobalamin by the reaction of stearic acid with vitamin B_{12r} with electrochemically generated oxygen or oxygen radicals and V(III) as the reductant. All compounds synthesized in this manner were identified by TLC and comparison of the R_f values with the same compounds synthesized by reliable alternate methods. The optical absorption spectra were also run (see Figure 8). Table III shows the organocobalamins that have been prepared in studies with the ten acids listed in Table I, their R_f values, and the most convenient method of synthesis. Yields of the organocobalamins are also given in Table III, based on total cobalamin (typically 2.5 μmol), and for the best method of synthesis.

All organocobalamins listed in Table III are primary. In cases where secondary or tertiary alkylcobalamins would have been the expected products, none were detectable. That secondary alkylcobalamins nevertheless are formed as intermediates follows from the results given in Table IV in which the observed yields of hydrocarbons in the oxidation of four carboxylic acids are given from reactions induced by V(III)/O_2, in the absence and presence of vitamin B_{12r}, before and after irradiation of the reaction solutions. This table shows that the yields of C_3H_6 from *i*-C_3H_7COOH generated in the dark increase substantially if vitamin B_{12r} is also present. However, upon subsequent irradiation of the reaction solution, no additional C_3H_6 is formed. This is in contrast to the behavior of *n*-C_3H_7COOH, where irradiation of the B_{12}-containing reaction solution causes a substantial release of C_3H_6 into the gas phase. Since isopropylcobalamin has too short a lifetime in solution[1] it is undetectable under these experimental conditions.

Discussion

The observed decomposition of carboxylic acids in solutions containing V(III) salts provides examples for induced oxidation of organic compounds under reducing conditions: oxygen radicals ($O_2^{-}\cdot$, $HO_2\cdot$, or $HO\cdot$), generated from O_2 on interaction with the reducing metal ions, are the actually reactive species. Interaction of these metal ions or aquated complexes thereof with oxygen may be schematically represented in terms of the equation

$$M^{n+} + O_2 \rightleftarrows [MO_2]^{n+} \rightarrow M^{+n+1} + O_2^{-}\cdot \qquad (3)$$

This equation only describes the formation of the superoxide ion and is oversimplified. The overall process of oxygen activation is far more complicated and involves pH-dependent reversible and irreversible processes leading to the formation also of H_2O_2, $HO_2\cdot$, and $HO\cdot$ and to more extensive oxidation of the metal ion, accompanied by the formation of M=O

Table IV. Yields of Hydrocarbons from the Oxidative Degradation of Four Carboxylic Acids Induced by V(III)/O_2 in the Absence and Presence of Vitamin B_{12r} in the Dark and after Irradiation of the Reaction Solutions

acid	B_{12r}	$h\nu$	obsd yields, nmol in gas phase[a] CH_4	C_2H_6	C_2H_4	C_3H_8	C_3H_6
none	+	−	7		4		
	+	+	10		4		
CH_3COOH	−	−	217				
	+	−	46	1	2		
	+	+	755	22	2		
C_2H_5COOH	−	−	6	108			
	+	−	12	5	800		
	+	+	12	12	1045		
n-C_3H_7COOH	−	−	11	13		106	86
	+	−	10		7	5	673
	+	+	10		13	9	933
i-C_3H_7COOH	−	−	16			93	5
	+	−	7			19	669
	+	+	7			23	684

[a] Reaction conditions: see legend to Table I, except that vitamin B_{12a} (7 μmol) was added to reaction solutions where indicated (+). All experiments were performed in the dark. Upon completion, the vitamin B_{12} containing solutions were exposed to a 160-W GE projector spot incandescent lamp at 30-cm distance.

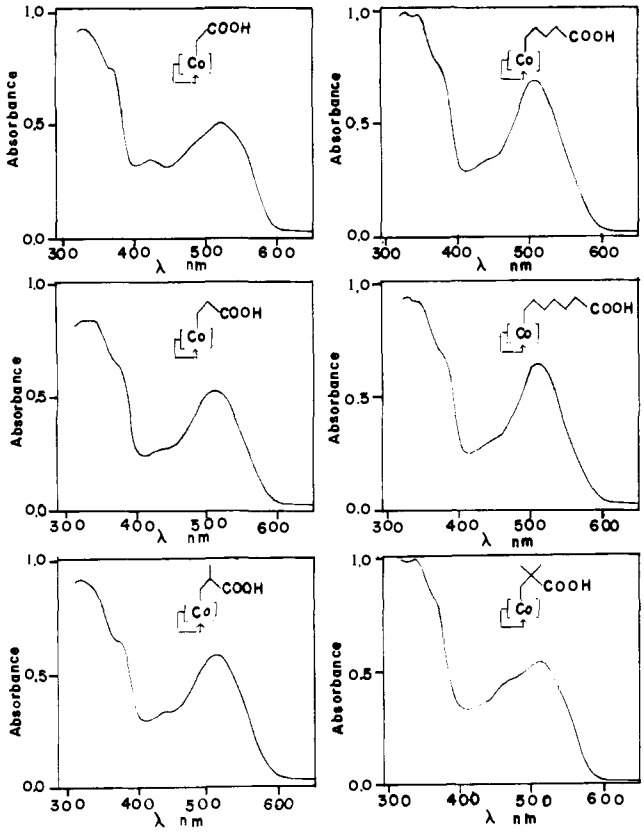


Figure 8. Optical absorption spectra of six ω-carboxyalkylcobalamins in 0.1 M phosphate buffer (pH 7.0).

bonds. The pH profile of the decomposition of CH_3COOH into CH_4 and CO_2 (see Figure 2) is indicative of this complexity. The first maximum at pH 1.6 suggests that under these conditions O_2 reacts with V^{3+}(aq). At pH 4.5, the position of the second maximum, the principal reacting species may be $V(OH)_2^+$, in keeping with the known behavior of V(III) ions on hydrolysis.[10] In acidic solutions of V(IV), the main ions present may be VO^{2+} or $V^{IV}O_x$ (x = 3 or 4).[11] These ions resist oxidation by air significantly in solution below the pH of 2.45. Above this pH, the rate of oxidation by air increases rapidly. In alkaline solutions, V(IV) exists as the ion VO_3^{2-} and has been shown to reduce oxygen rapidly to H_2O_2.[12] Since the second pH maximum for reactions with V(III) in Figure 2 coincides almost with that with V(IV) it is possible that both involve the same species. A detailed discussion of the mechanism of the vanadium–oxygen interactions is not within the scope of our work, however, especially since oxygen is not specifically required and can be replaced by H_2O_2.

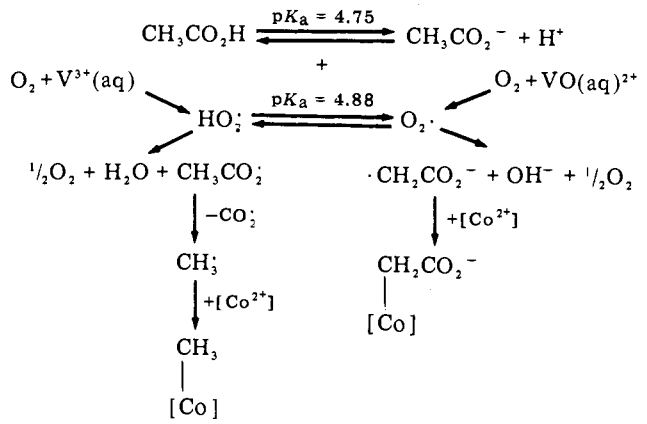
Reactions with Acetic Acid. The oxidation of carboxylic acids by oxygen radicals is known to be accompanied by decarboxylation and hydrogen-abstraction reactions which are strongly pH dependent. Acetate, a commonly used buffer, is not stable in solutions of V(III) salts in the presence of oxygen and undergoes decarboxylation and dehydrogenation reactions to yield $CH_3\cdot$ and $\cdot CH_2COO^-$ radicals, respectively. Owing to the presence of V(III) ions, $CH_3\cdot$ radicals are reduced to yield CH_4, which appears in the gas phase; the $\cdot CH_2COO^-$ radicals are also reduced, but this reaction does not afford a new product and merely prevents the formation of the radical coupling product succinate.

Our work shows that it is possible to intercept both $CH_3\cdot$, $\cdot CH_2COO^-$, and other organic radicals generated from carboxylic acids with vitamin B_{12r} to yield alkyl- and carboxyalkylcobalamins.[13] This is possible since we have found conditions under which vitamin B_{12r} is not irreversibly oxidized or degraded even though oxygen radicals are generated. Accordingly, the organocobalamins **1** and **2** are formed with acetic acid or acetate as the substrate. Since **1** is produced in significant yields only in *acidic* solutions, we conclude that oxidation of CH_3COOH to $CH_3COO\cdot$ occurs with $HO_2\cdot$ or $HO\cdot$ radicals. The dehydrogenation of CH_3COO^- to $\cdot CH_2COO^-$, on the other hand, is favored at higher pH and thus presumably involves the anion $O_2^{-}\cdot$. The relative yields of **1** and **2** thus are controlled by protic equilibria, the most important of which are outlined in Scheme I.

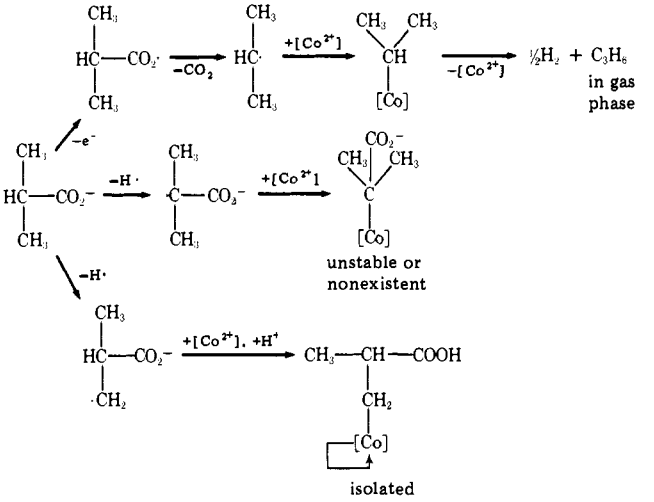
Scheme I is oversimplified inasmuch as it does not include H_2O_2 as the oxidant or OH radicals as reactive species. However, it satisfactorily describes the key reactions leading to the formation of **1** and **2**.

Reactions with Higher Carboxylic Acids. The oxidation of higher carboxylic acids is known to produce radicals which may either decarboxylate or undergo secondary reactions such as dimerization or further oxidation to aldehydes, etc.[5b] Oxidation of acids with Fenton reagent, for example, gives rise to the formation of CO_2 and the lower aldehyde(s), as well as to radical dimerization products.[5] Under our "oxidizing–reducing" conditions the radicals generated are less subject to further oxidation and are instead reduced, causing the formation of hydrocarbons rather than aldehydes, and radical

Scheme I. Formation of Methylcobalamin and of Carboxymethylcobalamin from Acetic Acid and Vitamin B_{12r} in Vanadium–Oxygen Systems



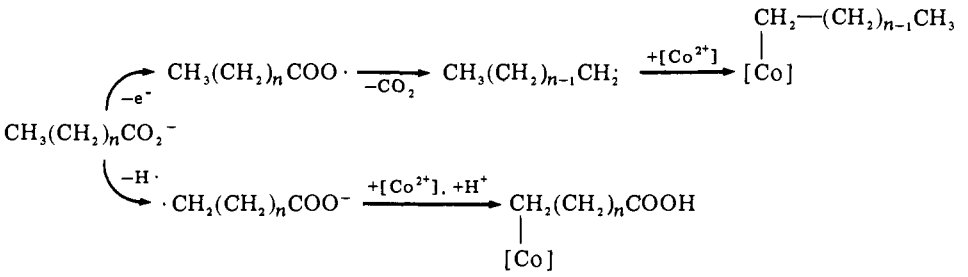
Scheme II. Oxidation of Isobutyric Acid in the Presence of Vitamin B_{12r}



coupling products; i.e., dicarboxylic acids are not formed, at least not under the experimental conditions employed thus far. In the presence of vitamin B_{12r}, the primary, secondary, and tertiary radicals generated are scavenged to yield the corresponding organocobalamins. Of these, however, only the primary organocobalamins are sufficiently long-lived to be isolable. The extraordinary selectivity of our reactions thus is explained by the fact that the Co(II) ion in vitamin B_{12r} is in a sterically hindered environment and that secondary or tertiary alkylcobalamins are unstable or capable of existence only under special conditions of reaction.[1]

The reactions of primary radicals generated from higher carboxylic acids may be formulated in terms of eq 4. The reaction with *isobutyric acid* as the substrate may serve as an instructive example for the behavior of a branched aliphatic carboxylic acid. Nonselective attack by oxygen radicals should give rise to the different radicals outlined in Scheme II. Decarboxylation of $(CH_3)_2CHCO_2\cdot$ would generate the isopropyl radical which on subsequent reaction with vitamin B_{12r} would yield isopropylcobalamin, a compound known to be unstable under the reaction conditions.[1] Abstraction of the α-hydrogen atom of isobutyric acid yields the radical $(CH_3)_2\dot{C}CO_2^-$, whose reaction with vitamin B_{12r} would produce a tertiary organocobalamin which would also be unstable. The one organocobalamin that could form and is stable would result from the reaction of vitamin B_{12r} with the radical $\cdot CH_2$-$CH(CH_3)COOH$, the only organocobalamin which is in fact detected. Hence, the apparently selective "ω-oxidation" of linear chain saturated fatty acids is in reality a sequel of the instability or short lifetime of secondary and tertiary alkylcobalamins.

Perhaps the most striking result of our present study is that organocobalamins are formed in high yields by simply shaking buffered solutions of vitamin B_{12r} in acetate buffer under reducing conditions in the presence of traces of air. The failure of maintaining strictly anaerobic conditions in experiments involving vitamin B_{12r} may thus give rise to unexpected products. One reaction which may be mentioned in this context has recently been described by Schaefer et al.,[16] who noticed the formation of an acetonyl derivative of *N,N'*-ethylenebis(3-fluorosalicylideniminato)cobalt by a reaction of acetone with the oxygen-carrying cobalt chelate. The authors plausibly assumed that in this reaction OH radicals are produced from the O_2/cobalt complex which in turn abstract hydrogen from acetone as shown in eq 5. Since analogies have been drawn[16] between reaction eq 5 and certain vitamin B_{12} or coenzyme B_{12} dependent enzymatic reactions it must be pointed out that in eq 5 as well as in our reactions the organic substrates are activated by the highly reactive oxygen radicals. A similar driving force is lacking in the hypothetical free-radical mechanisms of the B_{12}-dependent enzymatic reactions operating under strictly anaerobic conditions. On the other hand, it is possible that processes occur in certain microaerophilic organisms in which vitamin B_{12r} acts as a scavenger of free radicals. Irrespective of the potential biological significance of our work we have since extended our studies to a wide variety of organic substrates and discovered new methods of synthesis of organocobalamins which will be described in forthcoming papers.



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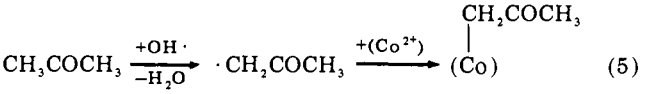

(5)

Experimental Section

Reagents and Chemicals. Vitamin B_{12a} (hydroxocobalamin, from Merck Sharp & Dohme Research Laboratories, Rahway, N.J.), vanadium(III)chloride (anhydrous, crystalline), and vanadyl sulfate (99.5%), both from Organic/Inorganic Chemical Corp., were used as received. All other inorganic reagents were of analytical or reagent grade quality and were also used without further purification. The carboxylic acids were available commercially from various sources and were purified by fractional distillation or recrystallization.

Stock Solutions. A stock solutions of V(III)-aq was prepared by dissolving 1.88 g of anhydrous VCl_3 in 30 mL of deaerated 1 M HCl

Table V. Oxidative Degradation of Acetic Acid in the Presence of Metal Ions[c]

ion added	yields of CH_4, μmol			
	V(III) absent		V(III) present	
	2[d]	4	2	4
none	0	0	3.9	3.0
Mo^{3+}[a]	0.75	0.75	4.7	4.4
Pd^{2+}	0.10	0.09	4.2	4.1
Fe^{2+}	0.03	0.08	3.7	3.0
Cu^{+}	0.03	0.04	3.3	3.1
Ti^{3+}	0.03	0.01	3.8	3.1
Cu^{2+}	0	0	0.6[b]	0.5[b]
Fe^{3+}	0	0	3.6	3.1
As^{3+}	0	0	0.4	0.5

[a] Added in form of K_3MoCl_6. [b] CH_3Cl detected by GLC. [c] Methane yields in the gas phase were measured after 6 days of reaction at 90 °C. Each metal ion was present at the concentration of 0.01 M. [d] Initial pH.

and stored under argon. A stock solution of VO^{2+} (aq) was prepared by dissolving 2.39 g of $VOSO_4 \cdot 2H_2O$ in 30 mL of deoxygenated 1 N H_2SO_4.

The stock solution of vitamin B_{12a} was always freshly prepared and contained 5 μmol/mL in deionized, distilled water.

Standard Gas-Chromatographic Techniques. The hydrocarbon products were identified and measured on a Varian Aerograph Model 2000 GLC instrument employing a 6-ft column packed with phenyl isocyanate on Porasil C (80–100 mesh) with helium as the carrier gas. The measurements were performed with flame-ionization detection at the column temperature of 40 °C. The identity of the hydrocarbons in question was confirmed by coinjection and by GLC–mass spectrography, using an LKB Model 9000 GC mass spectrograph. The hydrocarbon yields were determined by comparison of the peak areas with those of known amounts of the respective hydrocarbons. The calibration curves were prepared by withdrawing gas samples from bottles under the same conditions (i.e., in the presence of acetate buffer, and at the same temperature) as the actual experiments were run. Oxygen in the gas phase was analyzed by GLC using a Hewlett-Packard Model 700 laboratory gas chromatograph equipped with a 6-ft column filled with molecular sieve (5 Å).

Standard Techniques for Degradation of Acids under "Oxidizing–Reducing" Conditions. Except where indicated, all reactions were run in bottles of 38-mL capacity (obtained from Pierce Chemical Co., Rockford, Ill.). After capping with silicone rubber seals, the bottles were flushed with pure argon (99.99%, from Matheson). The sequence and amounts of reactants added were as follows and exemplified for acetic acid: glacial acetic acid (0.57 mL, 10 mmol), 10% NaOH solution (to adjust the pH), and deionized water to bring the final volume to 10.0 mL. The amount of NaOH solution was predetermined from prior tests. The solutions were deaerated by bubbling a stream of argon through the solutions for 1 h. The reactions were initiated by injecting 0.5 mL of the V(III) or −(IV) stock solutions, followed by a small amount of air or oxygen (usually 1–4 mL). The initial oxygen concentrations were measured by GLC. For experiments at very low initial concentrations of oxygen, traces of air were introduced during the addition of the reactants by using syringes that had not been previously flushed with argon.

For experiments at higher reaction temperatures, the bottles were placed into a water bath or into a thermostat-controlled drying oven. At different time points, gas samples were withdrawn for hydrocarbon analysis, The concomitant formation of CO_2 was not monitored but was demonstrated qualitatively by withdrawing gas samples from the reaction bottles after long reaction times and injecting them into a solution of $Ca(OH)_2$.

Effects of added metal ions were determined as described above, except that equivalent amounts of metal salts were added to the solutions. The reactions were run at two different pH values, both in the presence and absence of V(III). The results in Table V were obtained after 6 days of reaction at 90 °C.

Oxidative Degradation of Carboxylic Acids in the Presence of Vitamin B_{12r}. Reduction of vitamin B_{12a} with vanadium(III) and -(IV) ions was followed spectrophotometrically over a wide pH range under anaerobic conditions. One experiment is shown in Figure 5. With VO^{2+} (aq) the reduction of vitamin B_{12a} is slow at room temperature in acidic solutions but is rapid at neutral or alkaline pH. The reduction of vitamin B_{12a} with suspensions of $V(OH)_3$ in aqueous NaOH afforded vitamin B_{12s} which was identified by its characteristic absorption spectrum and reaction with alkylating agents.

Degradation of acids in the presence of vitamin B_{12r} was investigated by using the standard experimental technique described above except that usually 2.5 μmol of hydroxocobalamin was added. After termination of the reaction, the cobalamins were phenol extracted and isolated by precipitation with acetone/ether. The solid cobalamins were dissolved in a minimal amount of methanol and subjected to TLC on cellulose with the two solvent systems given in Table I. Isolated fractions of the cobalamins were identified by comparison of the R_f values and by cochromatography with authentic samples, as well as by optical absorption spectroscopy (see Table III and Figure 8). Identification of the compounds was achieved by comparison with authentic samples synthesized from the reactions of vitamin B_{12s} with the respective halogen derivatives (chlorides, bromides, or iodides, as available). Typically, 10 mg of hydroxocobalamin was dissolved in 5 mL of water and reduced to vitamin B_{12s} with 200 mg of $NaBH_4$ in a 38-mL reaction flask under exclusion of oxygen. After reduction of the vitamin was complete, solutions of the organic halogen compounds were added (usually at least a tenfold molar excess of alkylating agent was employed). After the reaction solutions attained the characteristic cherry-red color of alkylcobalamins, excess $NaBH_4$ was destroyed by the addition of 2 mL of acetone and the solutions were phenol extracted with 6 g of phenol and a few drops of CH_2Cl_2 (to facilitate phase separation). The cobalamins were isolated by precipitation with ether/acetone. A purity check was made by TLC and optical absorption spectroscopy. The presence of the Co-C bond in the molecule was demonstrated by recording the solution spectra before and after photolysis. Where necessary, the cobalamins were purified further by preparative TLC on cellulose.

Alternate Methods of Oxidation. In Table III the yields of products isolated by various methods of oxidation designated A, B, C, and D are indicated. Method A utilizes V(III) with O_2 as the oxidant and has been described above.

Method B uses Fenton reagent as the radical generator. The reactions were carried out in Erlenmeyer flasks of 10-mL capacity. As a rule, 5 mmol of the carboxylic acid and 2.5 μmol of vitamin B_{12a} (corresponding to 0.5 mL of the B_{12a} stock solution) were added into the flask, followed by 0.5 mL of the V(III) stock solution. After deaeration, 0.5 mL of a solution of $FeSO_4$ in 1 N H_2SO_4 (200 μmol) was injected. In the experiments with the lower carboxylic acids (acetic, propionic, *n*- and isobutyric, as well as pivalic) 1 mL of a 0.1% H_2O_2 solution was added very slowly over a period of 15 min, during which the solution was stirred as vigorously as possible (magnetic stirring was usually employed). For valeric, hexanoic, heptanoic, malonic, and succinic acid, 1 mL of 0.3% H_2O_2 was added in the course of 45 min. Note: Some acids are only partially soluble under these conditions, but this does not require a modification of experimental procedure in the cases studied.

Method C involves solutions of V(III) salts in alkaline solutions with O_2 as the oxidant. Enough 10% NaOH was added to the respective carboxylic acids to bring the pH to 13.5 (usually 6–7 mL of the NaOH solution was required to reach this pH in the presence of 10 mmol of the acids). Subsequently, 0.5 mL of VCl_3 stock solution and 0.5 mL of vitamin B_{12a} stock solution were added and the total solution volume was brought to 10 mL by the addition of distilled water. These solutions were stirred under air or in an O_2 atmosphere for 7–10 min, and organocobalamins (and unreacted vitamin B_{12}) were extracted by addition of 4 g of phenol and precipitated with ether/acetone. After isolation of the cobalamins through centrifugation they were separated by preparative TLC using water-saturated 2-butanol + 1% CH_3COOH as the ascending phase on cellulose plates.

Method D employed electrochemically generated oxygen or oxygen radicals under the same conditions as outlined for method C except that the reaction flasks were equipped with two Pt-wire electrodes and the solutions electrolyzed (without diaphragm) at 4.5 V and 30–50 mA. Electrolysis was carried out from 10–60 min; higher carboxylic acids need longer electrolysis times to afford good yields of the organocobalamin products. During electrolysis, a steady stream of pure argon was bubbled through the magnetically stirred reaction solutions. The synthesis of carboxymethylcobalamin will be described in greater detail. An Erlenmeyer flask of 10-mL capacity was equipped with Pt wire electrodes. Into this flask were added glacial acetic acid (0.57

mL, 10 mmol), 2.5 μmol of vitamin B_{12a} (0.5 mL of stock solution), 0.5 mL of V(III) stock solution, and 8.0 mL of 10% NaOH. The initial pH of this solution was 13.5. After deaeration with pure argon, the solution was electrolyzed as described above for 7 min, during which the conversion of vitamin B_{12} into carboxymethylcobalamin was essentially quantitative, as evidenced by TLC analysis of the phenol-extracted and ether/acetone precipitated cobalamin fractions. No yellow corrin oxidation products or methylcobalamin could be detected. The conversion of vitamin B_{12} to carboxymethylcobalamin was only 80% at pH 13.2 and fell to 35% at the pH of 12.6. At pH 11.2, the yield was only 6% after 10 min of electrolysis under otherwise identical conditions.

Synthesis of Carboxymethylcobalamin by the Use of Oxygen Gas or Air. Method D described above can be modified by bubbling oxygen or air through the reaction solution instead of electrolysis. After 7 min of reaction at the pH of 13.5, the yield of carboxymethylcobalamin was 99-100%, based on total vitamin B_{12}.

Preparative Synthesis of Carboxymethylcobalamin. Glacial acetic acid (0.57 mL, 10 mmol), a solution of 16.25 μmol (25 mg) of vitamin B_{12a} in 6.5 mL of water, 5.7 mL of a 10% aqueous solution of NaOH, and 0.5 mL of the V(III) stock solution were successively injected into a serum-capped, air-filled reaction bottle of 38-mL capacity, The bottle was gently shaken for 50 min at room temperature in the dark. The reaction solution was subsequently extracted with 10 g of phenol and the cobalamins precipitated with acetone/ether. Carboxymethylcobalamin was separated from unreacted vitamin B_{12a} and other byproducts by preparative TLC on cellulose plates. Carboxymethylcobalamin was isolated in 95% yield (based on vitamin B_{12a}); the remainders were side-chain-hydrolyzed carboxymethylcobalamin (1.7%) and unreacted hydroxocobalamin (3.3%).

Preparative Synthesis of Methylcobalamin from Acetic Acid. Glacial acetic acid (2 mL, 35 mmol) and 0.5 mL of a stock solution corresponding to 2.5 μmol (4.0 mg) were placed into an Erlenmeyer flask of 10-mL capacity which was equipped with a small magnetic stirrer bar and a silicone rubber seal with an argon inlet and outlet. The solution was deaerated by bubbling argon through the solution while stirring continued for 15 min. After deaeration, 0.5 mL of a solution of $FeSO_4$ in 1 N H_2SO_4 (200 μmol) and 0.5 mL of a solution of VCl_3 in 1 N HCl (200 μmol) were injected by means of a syringe. The reaction was started by the slow, dropwise addition of a 0.1% H_2O_2 solution. A total of 1 mL of this solution was added over the period of 15 min, during which the reaction mixture was stirred as vigorously as possible. The terminal pH was 1.6.

After completion of the addition of H_2O_2 the organocobalamins were isolated by phenol extraction and precipitation with ether/acetone. After separation by TLC on cellulose, methylcobalamin was isolated in 75.9% yield. Carboxymethylcobalamin was formed in 9.1% yield; unreacted vitamin B_{12} was present in 15% yield and recovered as B_{12a}. No yellow corrin oxidation products were detected.

Acknowledgments. This work was supported by Grant CHE 76-10890 of the National Science Foundation.

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(13) That vitamin B_{12r} can react with CH_3· and other radicals is well-known.[9a,14] Judging from the results of model experiments with other Co(II) chelates,[15] the rates of Co-C bond formation are high, with second-order rate constants in the other of $k_2 \sim 10^8\ M^{-1}\ s^{-1}$. Vitamin B_{12r} thus should be a very efficient radical scavenger, especially since the corrin ligand in addition is relatively more resistant against H abstraction by CH_3· radicals than a variety of other ligands of vitamin B_{12} model compounds.[14]
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Sterically Induced, Spontaneous Dealkylation of Secondary Alkylcobalamins Due to Axial Base Coordination and Conformational Changes of the Corrin Ligand[1]

John H. Grate[2] and G. N. Schrauzer*

Contribution from the Department of Chemistry, University of California at San Diego, Revelle College, La Jolla, California 92093. *Received November 20, 1978*

Abstract: The synthesis of various previously inaccessible secondary alkyl- and cycloalkylcobalamins by the reactions of olefins, alkyl iodides, and bromides with *hydridocobalamin* is described. The coordination of the axial 5,6-dimethylbenzimidazole ligand of alkylcobalamins is dependent on the steric bulk of the alkyl moiety and most secondary alkylcobalamins exist predominantly in the "base-off" form in neutral solution. Secondary and higher primary alkylcobalamins undergo sterically induced spontaneous dealkylation by way of syn β-elimination, the reverse of the reaction of hydridocobalamin with nonactivated olefins. They are generally more stable in acidic media, in which the axial base is protonated, while in neutral or alkaline solution axial coordination of this base causes a conformational change of the corrin ligand which accelerates the Co-C bond cleavage by orders of magnitude.

Whereas primary alkylcobalamins are as a rule reasonably stable compounds and hundreds of them have been prepared by many investigators since the early 1960s,[3] *secondary* alkylcobalamins seemed extremely labile and difficult to synthesize. This was attributed to steric restrictions imposed by the corrin ligand, since secondary alkyl derivatives of cob-

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