Synthesis and Reactions of Organocobalamins Relevant to the Mechanism of the Methylmalonyl-CoA–Succinyl-CoA Mutase Enzyme[1]

John H. Grate,[2a] Jay W. Grate,[2b] and Gerhard N. Schrauzer*

Contribution from the Department of Chemistry, University of California at San Diego, Revelle College, La Jolla, California 92093. *Received March 9, 1981*

Abstract: The synthesis of organocobalamins is described in which the cobalt atom carries substituents derived from esters of dimethylmalonic and methylmalonic acid. These compounds were previously known and studied only as transient intermediates in situ. They are of interest primarily as models of possible intermediates of the coenzyme B_{12} dependent methylmalonyl-CoA mutase reaction and have now been isolated for the first time. As typical representatives of sterically hindered primary organocobalamins, they were found to decompose spontaneously in neutral aqueous solution. The dimethylmalonate derivatives with substituents of the type $CH_2C(CH_3)(COOR)_2$ attached to cobalt lack hydrogen in the β position. They spontaneously decompose in neutral aqueous solution predominantly by a sterically induced homolytic cleavage of the Co–C bond. the methylmalonate derivative with $CH_2CH(COOR)_2$ attached to cobalt spontaneously decomposes by β elimination. Under conditions of Co–C bond homolysis, rearranged products from dimethylmalonic acid derivatives (methylsuccinic acid derivatives) are formed in low yields, if at all. High yields of rearranged products are generated if the respective cobalamins are subjected to reductive cleavage of the Co–C bond. A carbanionic mechanism of the methylmalonyl-CoA mutase reaction is discussed on the basis of these observations.

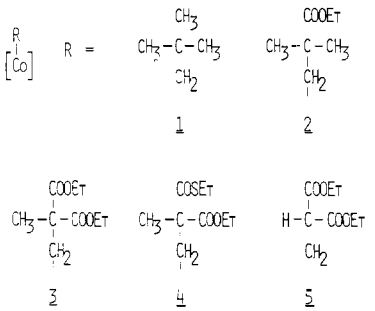
In recent papers[3,4] we described the preparation and properties of secondary and sterically hindered primary alkylcobalamins (Figure 1) which undergo spontaneous dealkylation in neutral aqueous solutions. We found that bulky alkyl groups in the "upper" position cause a "downward" distortion of the corrin ligand. This weakens the coordinative Co–N bond of the cobalt atom with the appended 5,6-dimethylbenzimidazole (DMBZ), forcing many of these cobalamins to exist in the "base-off" form. Co–C bond cleavage occurs when the axial DMBZ reattaches itself to the cobalt atom, as this is accompanied by an "upward" distortion of the corrin ligand.

With the unsubstituted alkylcobalamins studied thus far, two modes of spontaneous dealkylation have been observed: Syn β elimination, producing Co(I) and olefins, is favored when the alkyl group carries hydrogen in the β position to cobalt, as in isopropylcobalamin; homolytic Co–C bond cleavage (with efficient recombination to the parent organocobalamin under anaerobic conditions) occurs when no β hydrogen is present, as in neopentylcobalamin.

The two mechanisms can be distinguished, inter alia, by their different degree of oxygen sensitivity. Whereas spontaneous decomposition rates and olefin yields in the pure concerted syn-β-elimination reactions are unaffected by oxygen, a significant effect of oxygen is observed in the homolytic reactions because O_2 efficiently oxidizes the organic radicals produced and thus prevents their recombination with the vitamin B_{12r} present.

In the present paper, we examine the properties of the substituted organocobalamins **2–4**, which all are related to neopentylcobalamin, **1**, in that they lack β-hydrogen. In addition, cobalamin **5** was studied, which contains a β-hydrogen atom in place of a methyl group.

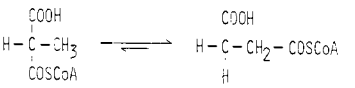
Cobalamins **2–5** contain ester or thioester moieties. Whereas the parent carboxylic acid of **2** has been described,[5] the ethyl ester is a new compound. Cobalamins **3**, **4**, and the dimethyl ester analogue of **5** have to date only been obtained in situ and were examined[6-8] as models of a potential intermediate in the coenzyme



1 **2**

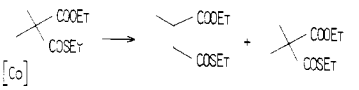
3 **4** **5**

B_{12} dependent enzymatic interconversion of methylmalonyl-CoA and succinyl-CoA (eq 1).



(1)

In each case, the cobalamins were previously[6-8] prepared in situ from vitamin B_{12s} and the respective organic bromide. They were unstable under the synthesis conditions and thus had to be studied directly in the synthesis solutions without prior isolation. From the decomposition of the dimethyl ester analogue of **5**, minor amounts of succinic acid were isolated in addition to methylmalonic and malonic acid. Similarly, cobalamin **3** yielded 1–5% diethyl succinate in addition to diethyl malonate. From cobalamin **4**, the rearranged product was predominant and resulted from exclusive thioester group migration, in analogy to the enzymatic reaction (eq 2).



(2)

However, as these reactions proceeded in the organocobalamin synthesis solutions, ambiguities remained as to the mechanisms of Co–C bond cleavage. Since the reaction solutions contained excess reducing agent ($NaBH_4$), it was not possible to decide whether the Co–C bond cleavage was spontaneous or reductive. Accordingly, no firm conclusions concerning the mechanism of the ensuing rearrangements were possible. Since our recognition of the conditions necessary for the synthesis and isolation of sterically labile organocobalamins,[3,4] we decided to isolate the cobalamins **2–5** and to study their reactions under various con-

(1) Paper No. 53 of a series "Studies on Vitamin B_{12} and Related Compounds".

(2) (a) Catalytica Associates, Inc., Santa Clara, CA 95051. (b) National Science Foundation Predoctoral Fellow, 1978–1981.

(3) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541.

(4) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601.

(5) Schrauzer, G. N.; Hashimoto, M. *J. Am. Chem. Soc.* **1979**, *101*, 4593.

(6) Retey, J. In "Vitamin B_{12}"; Zagalak, B.; Friedrich, W. Eds., Walter de Gruyter: Berlin, 1979; pp 439–460.

(7) (a) Scott, A. I.; Kang, K. *J. Am. Chem. Soc.* **1977**, *99*, 1997. (b) Scott, A. I.; Kang, J.; Dalton, D.; Chung, S. K. *Ibid.* **1978**, *100*, 3603. (c) Scott, A. I.; Hansen, J. B.; Chung, S. K. *J. Chem. Soc., Chem. Commun.* **1980**, 388.

(8) Dowd, P.; Shapiro, M. *J. Am. Chem. Soc.* **1976**, *98*, 3726.

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Table I. Spectral Data of Organocobalamins Synthesized[a]

	2	3	4	5
λ		pH 7		
λ_{max}, nm (ε)	330 (15200)	332 (15300)	332 (16400)	336 (14200)
	377 sh (8540)	372 sh (9300)	370 sh (9880)	373 (11400)
	430 (6090)	418 sh (5500)	412 (5750)	429 (5130)
	507 (7590)	516 (7870)	516 (8330)	522 (9080)
λ_{min}, nm (ε)	413 (5930)	444 (5280)	445 (5090)	410 (4880)
		pH 2		
λ_{max}, nm (ε)	324 (15500)	324 (15700)	324 (17000)	325 (15400)
	423 (8490)	346 sh (13600)	344 sh (15300)	406 (7350)
		416 (9520)	420 (9880)	459 (8490)
		447 sh (8690)	451 (8930)	
λ_{min}, nm (ε)	378 (8280)	384 (7510)	385 (7190)	387 (7100)

[a] In 10 mM sodium phosphate buffers.

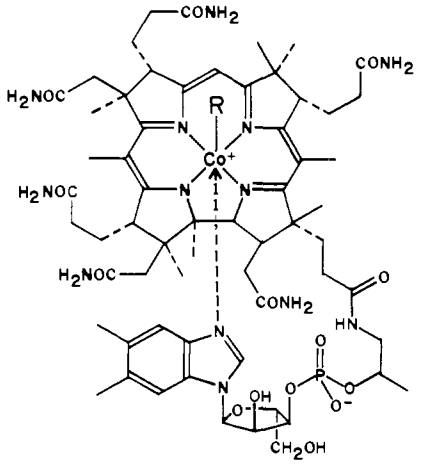
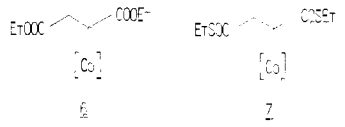


Figure 1. The structure of alkylcobalamins in the base-on form. Cobinamides lack the axial base and phosphoribosyl moieties.

trolled conditions. In addition, the potential of cobalamins such as **6** and **7** to effect the reverse rearrangement to methylmalonate derivatives was investigated.



Results

Synthesis and Reactions of Organocobalamins 2–4. The neopentyl-like cobalamins **2–4** were obtained by the reaction of vitamin B_{12s} with the appropriate organohalide in methanol containing 10% (w/v) NH_4I. The ammonium salt serves to protonate the axial DMBZ, allowing the isolation of the cobalamin in the protonated base-off form. Metallic zinc was used as the reducng agent to generate vitamin B_{12s} in preference to the commonly employed $NaBH_4$, primarily because zinc can be readily removed by centrifugation after completion of the reaction. The base-off protonated organocobalamins were isolated by precipitation with acetone, as is outlined in the Experimental Section. The corresponding cobinamides **3** and **4** are sufficiently stable in aerobic neutral solution to be prepared and isolated by conventional methods.

In neutral solutions, cobalamins **2**, **3**, and **4** exist predominantly in the base-on form, as evidenced by their absorption spectra and their red color in solution. In acidic solution, the base-off protonated forms of the cobalamins are yellow and exhibit spectra identical with those of the corresponding cobinamides. Spectral data are given in Table I.

On exposure to visible light in neutral aqueous solutions under strictly anaerobic conditions, cobalamins **2**, **3**, and **4** undergo photolysis very slowly, requiring hours of irradiation to effect net Co–C bond cleavage. Vitamin B_{12r} is formed in the process.

Table II. Observed Rates of Spontaneous Aerobic Decomposition of Related Organocobalamins and -cobinamides in Neutral Solution[a]

organocorrin	k_{obsd}	$t_{1/2}$, min
neopentylcobalamin	1.5×10^{-4}	75
cobalamin 2	1.6×10^{-4}	74
3	1.4×10^{-4}	83
4	2.2×10^{-4}	52
5	2.8×10^{-5}	407
neopentylcobinamide	1.1×10^{-7}	73[b]
cobinamide 3	8.6×10^{-7}	9.4[b]
4	1.0×10^{-6}	7.7[b]

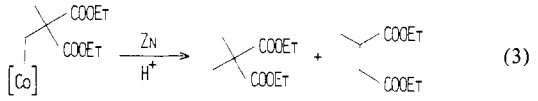
[a] In 0.1 M/pH 7.0 sodium phosphate buffer; measurement temperature, 25 °C. [b] Half-life measured in days.

Aerobic photolysis is complete within a few minutes and yields vitamin B_{12a}. This behavior is reminiscent of that of neopentylcobalamin.[3] In the dark, neutral aqueous solutions of these cobalamins exhibit a sensitivity to oxygen which is also similar to that of neopentylcobalamin. When the solutions are stored under rigorously anaerobic conditions, a slow decomposition is noted but is incomplete even after many weeks. Under aerobic conditions, neutral aqueous solutions are very unstable; the first-order decompositions to vitamin B_{12a} and oxidation products of the organic residues occur with room-temperature half-lives of only 1–2 h. When a neutral solution of cobalamin **3** was exposed to air after several days of anaerobic dark storage, the remaining intact organocobalamin decomposed to vitamin B_{12a} at the same rate as freshly prepared samples. This suggests that no new stable organocobalamins were formed during the anaerobic dark storage. Rates of aerobic decomposition in neutral aqueous solution are given in Table II with those of cobinamides **3** and **4**. Under the same conditions, the latter have half-lives of over a week. In acidic solution, the cobinamides and base-off cobalamins decompose at similar rates but generally more rapidly than the cobinamides in neutral solution, presumably due to secondary reactions possibly involving protic reactions of the esters. These reactions were not followed in detail; they are not within the scope of this paper.

Aerobic decomposition of cobalamin **3** in methanol afforded diethyl methylmalonate as the only detectable product. Aerobic decomposition of **4** in 1:3 (v/v) methanol/pH 9 borate buffer likewise produced some *O,S*-diethyl methylthiomalonate along with traces of *O,S*-diethyl dimethylthiomalonate and predominantly an unidentified (presumably oxidized) product. Prolonged irradiation of **4** in anaerobic 1:3 (v/v) methanol/pH 9 borate buffer produced *O,S*-diethyl dimethylthiomalonate and rearranged *O,S*-diethyl 2-methyl-4-thiosuccinate in the rearranged:unrearranged ratio 1:20.

Anaerobic reductive cleavage of the Co–C bond can be effected by zinc in methanol containing 5% (w/v) NH_4Br. From cobalamins **3** and **4**, vitamin B_{12s} is formed quantitatively within 30 min. The organic products thus obtained from cobalamin **3** are diethyl dimethylmalonate and the rearranged diethyl methyl-

succinate in the rearranged:unrearranged ratio 1:100 (eq 3). This



(3)

100 : 1

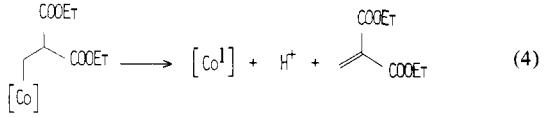
result is in close agreement with the 1–5% yield of rearranged product reported from the in situ experiments.[6]

The zinc-induced reductive cleavage of cobalamin **4** produces *O,S*-diethyl 2-methyl-4-thiosuccinate and *O,S*-diethyl dimethylthiomalonate in a 25:1 ratio. Similar results (rearranged:unrearranged = 30:1) were achieved when *O,S*-diethyl (bromomethyl)methylthiomalonate was reduced catalytically with excess zinc and catalytic amounts of vitamin B_{12} in methanol containing 5% (w/v) NH_4Br. Exclusive thioester migration was confirmed by the ^1H NMR spectrum of the isolated rearranged product. When vitamin B_{12} is absent, zinc slowly reduces *O,S*-diethyl (bromomethyl)methylthiomalonate directly, giving rearranged and unrearranged products in a 1:9 ratio.

Reductive cleavage of these cobalamins is also effected by sodium borohydride. Reaction of cobalamin **4** with $NaBH_4$ in strictly anaerobic 1:3 (v/v) methanol/pH 9 borate buffer afforded rearranged and unrearranged products in the ratio of 23:1, virtually the same as the ratio achieved by the reductive cleavage with zinc. Under aerobic conditions, the rearranged:unrearranged ratio effected by the action of borohydride on cobalamin **4** prepared in situ was only 2:1, which more closely approximates the results of ref. 7.

Synthesis and Reactions of Cobalamin 5. Cobalamin **5**, 2,2-bis(ethoxycarbonyl)ethylcobalamin, is sufficiently stable in the base-on form to survive preparation in neutral solutions. It was prepared by reducing vitamin B_{12a} with zinc in the presence of tetraethylammonium bromide, using diethyl (bromomethyl)-malonate as the alkylating agent. After removal of the zinc, acetone precipitation from the methanolic solution afforded the solid product in the base-on form. However, extensive Co–C bond cleavage occurred in attempts to purify cobalamin **5** by means of phenol extraction or by precipitation with acetone from borohydride containing synthesis solutions, as has been reported for the dimethyl ester analogue.[8]

The spontaneous decomposition of cobalamin **5** in aerobic neutral solution produces vitamin B_{12a} in a first-order process with a half-life of 7 h. Vitamin B_{12s} is formed initially, as evidenced by the formation of ethylcobalamin when cobalamin **5** was allowed to decompose anaerobically in the presence of ethyl bromide. This behavior is reminiscent of sterically labile alkylcobalamins carrying β-hydrogen, which spontaneously form vitamin B_{12s} and olefins.[4] Accordingly, the initial organic product from cobalamin **5** is expected to be the highly reactive diethyl methylenemalonate (eq 4).



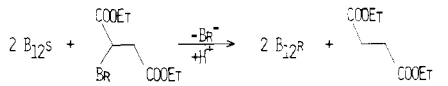
(4)

Traces of diethyl methylenemalonate were observed among the organic products isolated from the anaerobic spontaneous decomposition of cobalamin **5**. The main organic product from both the anaerobic and aerobic spontaneous decompositions, however, was a polymeric material having a ^1H NMR spectrum similar to that of the product obtained by injecting authentic diethyl methylenemalonate into buffered aqueous solutions. In addition, the reduced product, diethyl methylmalonate, is formed during the spontaneous decompositions of cobalamin **5** under anaerobic conditions. It must arise by a reductive cleavage reaction induced by the vitamin B_{12s} generated simultaneously by the β-elimination process.

Reductive cleavage of cobalamin **5** by $NaBH_4$ in anaerobic pH 9 borate buffer yielded diethyl methylmalonate as the sole observed product. In contrast to the behavior of cobalamin **3** and the dimethyl analogue of cobalamin **5**[8] under reducing conditions, we were unable to detect any diethyl succinate among the organic products.

Reactions of Fumarate and Bromosuccinate Esters with Cob(I)alamin. Cobalamins **6** and **7** proved too unstable for isolation. Green solutions of vitamin B_{12s} or cob(I)inamide turned brown immediately after the addition of the organic reactants, but only Co(II) (and no organocorrins) could be detected in the reaction solutions. Accordingly, only the reactions of cob(I)alamin with bromosuccinate or fumarate esters were studied.

The reaction of equimolar amounts of diethyl bromosuccinate with vitamin B_{12s} in the absence of other reducing agents produced diethyl succinate in 50% yield, based on total vitamin B_{12s}. Half of the alkylating agent remained unreacted, and no fumarate or maleate esters were detected (eq 5). Similarly, equimolar



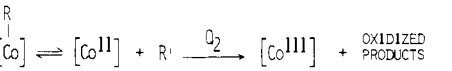
(5)

quantities of diethyl fumarate reacted with hydridocobalamin in 1:1 (v/v) ethanol/acetic acid to yield 50% diethyl succinate, with half of the diethyl fumarate remaining.

Stoichiometric reactions of the dithioester, *S,S*-diethyl dithiofumarate, with vitamin B_{12s} yielded vitamin B_{12r} and equimolar amounts of *S,S*-diethyl dithiosuccinate and *S,S*-diethyl dithiofumarate. No rearranged product, i.e., *S,S*-diethyl methyldithiomalonate, was observed.

Discussion

This study demonstrates that cobalamins **2–4** can be prepared and isolated by methods similar to those used to make the parent compound, neopentylcobalamin (**1**).[3] Cobalamins **2–4** also exhibit the same oxygen sensitivity as neopentylcobalamin in neutral aqueous solution, which is clearly indicative of sterically induced Co–C bond homolysis. Under anaerobic conditions, the rates of spontaneous decomposition are very slow, indicating that the recombination of the initially formed radicals occurs with high efficiency. No evidence has been obtained for the occurrence of skeletal rearrangements of the organic radicals prior to their recombination with vitamin B_{12r}. Accordingly, the spontaneous decomposition of the cobalamins **2–4** can be formulated in terms of eq 6.



(6)

The observed formation of demethylated products (methylmalonate esters) from the aerobic decompositions of **3** and **4** is most plausibly attributed to the retro-aldo elimination of formaldehyde from (hydroxymethyl)methylmalonate esters arising from the oxidized radical.

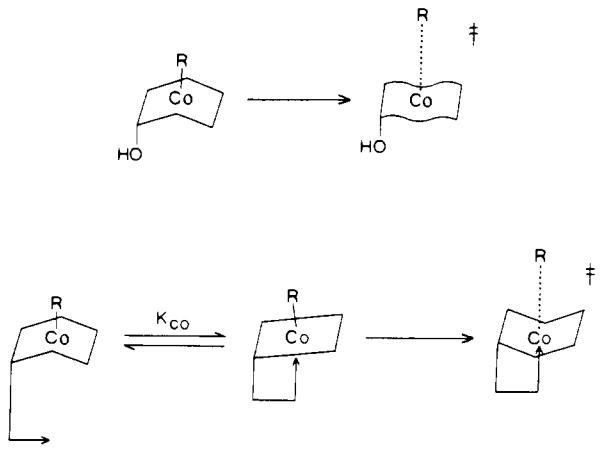
The cobinamides **3** and **4** are far more stable than the corresponding cobalamins in aerobic neutral solution. This confirms that these spontaneous homolyses are sterically induced.[3,4] Thermal motions of the corrin ligand are less efficient at promoting Co–C bond cleavage than upward conformational motions caused by the coordination of the axial DMBZ in the cobalamins (Scheme I).

Unlike neopentylcobalamin, which is mostly base-off in neutral aqueous solution ($K_{co} = 0.4$),[3] cobalamins **2–4** are predominantly base on ($K_{co} > 1$). This is attributable to the inductive effect of the ester substituents, which favor axial coordination of DMBZ.[9] Cobalamin **5**, with a less bulky organic group attached to cobalt, is predominantly base-on. Its aerobic spontaneous decomposition in neutral aqueous solution is slow and occurs predominantly by a β-elimination mechanism, affording vitamin B_{12s} and methylenemalonate as the initial products (see eq 4).

The formation of malonic acid from the decomposition of the dimethyl analogue of **5** has been observed by others[8] and may be attributed to the retro-aldol decomposition of hydroxymethyl-

(9) Hogenkamp, H. P. C.; Rush, J. E.; Swenson, C. A. *J. Biol. Chem.* **1965**, *240*, 3641.

Scheme I

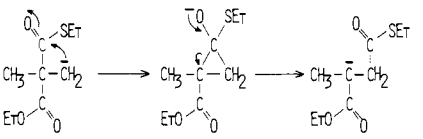


malonic acid, a compound which is likely to formed, e.g. by the addition of H_2O to methylenemalonate under the hydrolytic workup conditions employed.

In the presence of reducing agents such as zinc or $NaBH_4$, the Co–C bonds of cobalamins **3–5** are reductively cleaved. With alkylcobalamins and -cobaloximes, such reactions have been shown previously to yield alkanes through the protonation of intermediate alkylcarbanions.[10] Carbanions are also formed in the reductive cleavage of cobalamins **3–5**, giving rise to the formation of unrearranged and in some cases rearranged products. Thus, from the cobalamins **3** and **4**, mixtures of dimethylmalonate and methylsuccinate esters are formed.

Under our experimental conditions, the reductive cleavage leading to rearranged products is faster than the spontaneous aerobic and anaerobic homolysis. Moreover, the rearranged product from cobalamin **4** was observed in high yields *only* under reducing conditions and at a nearly constant ratio to unrearranged product, regardless of the reducing agent employed. This indicates that the rearrangement of the organic moiety does not occur as a radical or while bound to cobalt. Our results thus are consistent with those of the previous investigators, which have shown that radicals homologous[11] or identical[6] with those arising from Co–C bond homolysis of **3** do not rearrange.

Cobalamins **3** and **4** give markedly different rearranged to unrearranged product ratios under reducing conditions, even though they must have very similar Co–C bond stabilities. The thioester moiety of **4** evidently possesses far greater migratory aptitude than the ester group of **3**, a fact which provides further evidence that rearrangement proceeds from a carbanion as shown in eq 7.



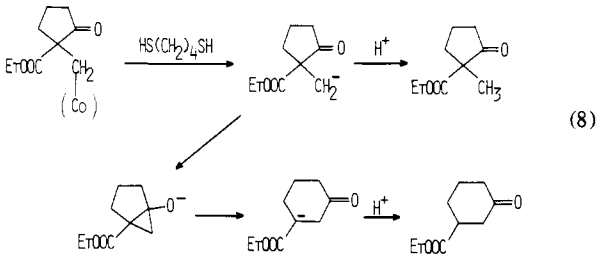
(7)

Thioesters are more electrophilic than esters and thereby more susceptible to intramolecular carbanionic attack, which must also compete with protonation of the initial carbanion. The reaction of *O,S*-diethyl (bromomethyl)methylmalonate with vitamin B_{12s} and $NaBH_4$ in ethanol-*d* was previously shown[7b] to result in deuterium incorporation into both unrearranged and rearranged products at the positions indicated by the structures of the above carbanions.[12] No deuterium incorporation from C_2D_5OH or $NaBD_4$ was observed.[7b]

Since rearranged product was still formed in these in situ experiments when the authors added acetone or acetaldehyde to destroy the excess $NaBH_4$,[7c] the *O,S*-diethyl (bromomethyl)-methylthiomalonate was proposed to be reduced by vitamin B_{12s} in a one-electron step to yield vitamin B_{12r} and free organic radicals. A portion in the radicals were then thought to rearrange, and both unrearranged and rearranged radicals were assumed to combine with vitamin B_{12r} to yield a mixture of organocobalamins which did not interchange. Spontaneous heterolytic cleavage of these organocobalamins was finally assumed to account for the observed deuterium incorporation.

This mechanistic proposal cannot be reconciled with our observations. We invariably noticed that the addition of acetone to borohydride-containing synthesis solutions of cobalamin **4** induced considerable Co–C bond cleavage, just as occurs for cobalamin **5** and its dimethyl ester analogue.[8] By isolating cobalamin **4** and investigating its modes of reaction in solution unambiguously free of reducing agents, we noticed that the ratios of rearranged to unrearragned products are *variable*, depending on the conditions of Co–C bond cleavage chosen. Thus, the rearrangement does not occur prior to Co–C bond formation. Furthermore, the clean first-order decomposition of pure cobalamin **4** to pure vitamin B_{12a} demonstrates that only one organocobalamin is present.

A carbanionic rearrangement mechanism for the methylmalonyl-CoA–succinyl-CoA mutase reaction was first proposed by Ingraham in 1964[13] and was later supported by the model reaction shown in eq 8,[14] after we had shown that carbanions can be generated from organocobaloximes on reaction with dithiols.[15]



(8)

In eq 8, the rearranged product resulted from intramolecular carbanionic attack on the ketone; no rearranged product resulting from intramolecular carbanionic attack on the less electrophilic ester function was observed. This parallels the observation that ester groups show only weak tendency to migrate. Ketones and thioesters, however, have similar electrophilicities.[16]

In another model reaction, the cobaloxime analogue of **3** was noted to produce rearranged product (methyl succinate) in variable irreproducible yields on photolysis[6] suggestive of a radical mechanism. Although the photolysis of the Co–C bond in organocobalamins is usually considered to produce only radicals, it must be pointed out that this is an oversimplification. Thus, in the anaerobic photolysis of methylcobalamin in neutral D_2O, the methane produced contains 66% CH_3D.[17] The CH_3D arises from the reduction of the CH_3 radicals to carbanions by cobalt(II), and these subsequently react with D^+ ions of the solvent. This reductive process should be favored if the group attached to cobalt carries inductively electron-attracting substituents.

Other studies have shown that when the dimethyl malonate moiety in the cobaloxime analogue of **3** is anchored to the equatorial ligands, the rearrangement proceeds with 80–90% efficienty on photolysis.[6]

In view of the evidence cited above, this rearrangement must proceed via reduction of the initial organic radical by Co(II) to a carbanion. Reduction of the radical is favored in this case because the initially formed radical pair is held close together. This hinders termination by H abstraction and restores the Co–C

(10) (a) Schrauzer, G. N.; Seck, J. A.; Beckham, T. M.; Holland, R. J.; Rubin, E. M.; Sibert, J. W. *Bioinorg. Chem.* **1972**, *2*, 93. (b) Schrauzer, G. N.; Seck, J. A.; Beckham, T. M. *Ibid.* **1973**, *2*, 211. (c) Schrauzer, G. N. *Pure Appl. Chem.* **1973**, *33*, 545.

(11) Lewis, S. N.; Miller, J. J.; Winstein, S. *J. Org. Chem.* **1972**, *37*, 1478.

(12) Multiple deuterium incorporation from ethanol-*d* into the rearranged product subsequently reported by the same authors[7c] may be ascribed to the acidity of the protons, α to the carbonyl groups.

(13) Ingraham, L. L. *Ann. N. Y. Acad. Sci.* **1964**, *112*, 713.

(14) Lowe, J. N.; Ingraham, L. L. *J. Am. Chem. Soc.* **1970**, *93*, 3801.

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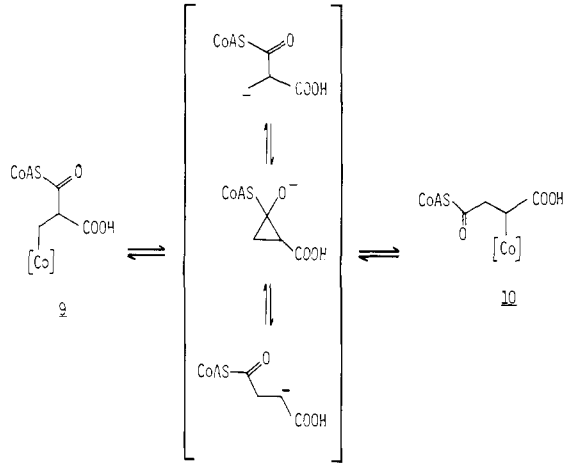
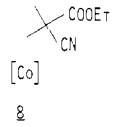
Scheme II



bond by recombination if reduction does not occur.

Similarly, radical reduction by vitamin B_{12r} accounts for the formation of a low yield of rearranged product when cobalamin **4** is photolyzed anaerobically.

This reduction–rearrangement mechanism can also account for the formation of rearranged product from 2-cyano-2-(ethoxycarbonyl)propylcobalamin (**8**).[6] Compared with **3** and **4**, the



organic group in **8** is sterically less demanding, rendering the cobalamin stable to conventional methods of purification.

Both cyano and ester group migration were observed from **8** after exposure to daylight in solution. However, cyano group migration is preferred over ester group migration; this is to be expected since the cyano group is intermediate in electrophilicity between the ester and thioester group and provides further evidence for rearrangement proceeding via a carbanionic species.

Incorporation of cobalamin **9** as an intermediate in the methylmalonyl-CoA–succinyl-CoA mutase reaction would require the specific inhibition of the β-elimination reaction in favor of a net heterolytic Co–C bond cleavage to a carbanion. Conceivably, β-elimination can be suppressed through interactions of the organocorrin intermediate with the enzyme protein. The net heterolytic cleavage of the Co–C bond in enzyme-bound **9** could occur directly or through interaction with a thiol or dithiol center in the vicinity of the active site. Since the mutase reaction proceeds without the net consumption of reducing equivalents,[18] all electron-transfer reactions must be reversible. If **9** is assumed to be the enzymic intermediate, microscopic reversibility requires the assumption that **10** is also an intermediate. The electron-transfer reactions must be of necessity internal, since the enzymatic reaction proceeds without the comsumption of external reductant (Scheme II).

Our work shows that the cobalamin analogues **6** and **7** decompose under our conditions by heterolysis to yield succinate esters. The observed stoichiometry of the reaction of vitamin B_{12s} with the alkylating agents employed is consistent with Scheme III. Fast reductive cleavage of the organocobalamin by vitamin B_{12s} would also give the observed stoichiometry.

Under the experimental conditions employed, no rearranged products, i.e., methylmalonate esters, were detected. Protonation of the carbanion is apparently favored over intramolecular reactions. In the enzymatic reaction, the equilibrium ratio of succinyl-CoA to methylmalonyl-CoA is 23:1,[19] indicating that the yield of methylmalonate esters should be low in any case.

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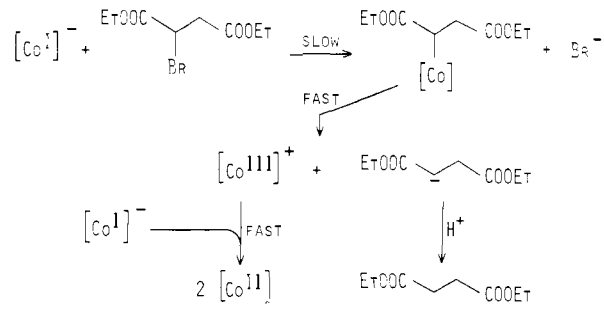
Scheme III



Recognition of the carbanionic nature of the rearrangement catalyzed by methylmalonyl-CoA mutase readily accounts for the intramolecular nature of the rearrangement and for the exclusive migration of the thioester moiety. Indeed, it is now clear that the biological role of CoA in this reaction is to create a site on the substrate which is sufficiently electrophilic to promote the intramolecular carbanionic reaction. It should be emphasized, however, that the rearrangement represents only a part of the enzymatic process. further aspects of the reaction remain to be elucidated, especially those related to the initial activation of the substrates on interaction with the enzyme.

Experimental Section

Materials. Vitamin B_{12a} and vitamin B_{12} (cyanocobalamin) were obtained from Merck, Sharp and Dohme Research Laboratories, Rahway, NJ. Diaquocobinamide was prepared from vitamin B_{12} via dicyanocobinamide according to published procedures.[20] Argon (Matheson) was freed of oxygen by passage through two Cr^{2+} scrubbers in series and dried with anhydrous calcium sulfate where necessary. Other reagents not discussed below were commercially available and used as received.

Methods and Instrumentation. Organocorrinoids were handled in dim light or in aluminum foil wrapped glassware. Optical absorption spectra were recorded on Beckman DK-2A recording spectrophotometer equipped with a temperature-regulated cell block. Photolyses of organocorrinoids were performed at 15-cm distance from a 150-W tungsten-filament floodlamp with air-stream cooling. Gas-chromatographic analyses were performed by using a Hewlett-Packard Model 700 gas chromatograph equipped with a 20 × $^1/_8$ in. column of Poropak R (Waters Associates) with He as the carrier gas and FID detection. By variation of the carrier gas flow and the column temperature, separations of various component mixtures were achieved. The identity of the products was verified by comparison with authentic samples and cochromatography. ^{1}H NMR spectra were determined on either a Varian EM-360 60-MHz NMR spectrometer or a Varian HR 220/Nicolet TT-100 spectrometer. Thin-layer chromatography (TLC) of organic products was done with silica gel 60-F254 (Merck), 0.25 mm or 2 mm, usually with 4:1 benzene:hexane as the ascending phase, unless otherwise specified. The TLC and GLPC methods used do not separate the two isomeric *O,S*-diethyl methylthiosuccinates.

Alkylating Agents and Standards. Ethyl 3-iodo-2,2-dimethylpropionate was prepared from 3-chloro-2,2-dimethylpropionic acid by acid-catalyzed esterification with ethanol followed by refluxing with NaI in methyl ethyl ketone. Diethyl (bromomethyl)methylmalonate was prepared by the reaction of diethyl methylmalonate with sodium ethoxide and CH_2Br_2 in ethanol. *O,S*-Diethyl dimethylthiomalonate and *O,S*-diethyl (bromomethyl)methylthiomalonate were prepared as outlined in ref 7a. *O,S*-diethyl methylthiomalonate was prepared analogously. A mixture of *O,S*-diethyl 2-methyl-4-thiosuccinate and *O,S*-diethyl 3-methyl-4-thiosuccinate was prepared by the reaction of methyl succinic anhydride with ethanol, followed by the esterification of the remaining carboxylic acid function with ethanethiol by procedures in ref 7a. The two isomers were not separated. Comparison of the 220-MHz spectrum of the product with the spectra of the individual isomers[7a] showed that both were present. Diethyl methylenemalonate was prepared according to Bachman and Tanner[21] as modified by Tagaki and Asahara.[22] The ester polymerizes at room temperature, affording a polymer which is soluble in

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CH_2Cl_2. Monomeric diethyl methylenemalonate was generated from the polymer by cracking at >200 °C at 1 atm. Diethyl (bromomethyl)-malonate was prepared according to Cannon.[23]

Diethyl bromosuccinate was prepared by the acid-catalyzed esterification of bromosuccinic acid with ethanol. The product must be distilled at low pressures (0.25 torr) to avoid decomposition to diethyl fumarate.[24] *S,S*-Diethyl dithiofumarate was prepared as described in ref 25. *S,S*-Diethyl dithiosuccinate was prepared by reducing *S,S*-diethyl dithiofumarate with Zn in freshly prepared 10% (w/v) NH_4I in absolute ethanol. *S,S*-Diethyl methyldithiofumarate was prepared by first reacting methylmalonic acid with thionyl chloride with pyridine as a catalyst. The resulting oil (after distillation) was esterified with ethanethiol by using the same procedure as applied for the synthesis of diethyl dithiofumarate. The product was purified by vacuum distillation and preparative TLC on silica gel eluted with benzene. The ^1H NMR spectra of all compounds synthesized were measured to confirm their identity and purity.

Preparation of Organocobalamins and -cobinamides. The organocobalamins **2–4** were prepared by dissolving 250 mg of hydroxocobalamin in 10 mL of freshly prepared 10% (w/v) NH_4I in absolute methanol in a 15-mL capacity centrifuge tube. The solution was deaerated by flushing with argon. Subsequently, 250 mg of Zn dust was added, and the tube was capped with a rubber septum. Argon flushing was continued via inlet and exit needles. When the formation of vitamin B_{12s} was complete (after ca. 1 min.), 0.10 mL of the alkylating agent was injected by means of a syringe. The reaction solutions turned orange immediately. After 1 min, the tube was centrifuged to remove the excess Zn. This is necessary because the prolonged exposure of the product to zinc results in net losses of organocobalamin due to the reductive cleavage of the Co–C bond. The supernatant was transferred via catheter under Ar pressure into 250 mL of deaerated acetone, giving the product as a yellow precipitate (NH_4I and Zn^{2+} salts both remain dissolved in the acetone supernatant). The product cobalamin was collected by centrifugation, washed twice with 35 mL of acetone and once with 35 mL of ether, and dried under a stream of dry argon. The solid products **2–4** are sufficiently stable to be handled in air. The purity of the samples was monitored by optical absorption spectroscopy.

Cobalamin **5** was prepared by reducing vitamin B_{12a} to vitamin B_{12s} with zinc dust in 5% (w/v) $(C_2H_5)_4N^+Br^-$ in CH_3OH. Under these conditions, the reduction to vitamin B_{12s} is slow and requires ca. 10 min. of vigorous shaking. After injection of the alkylating agent, the solution was worked up as described for the cobalamins **2–4**.

The cobinamides **3** and **4** were synthesized by dissolving 10 mg of diaquocobinamide in 1 mL of water in a septum-stoppered test tube. After deaeration of the solution with argon, 25 mg of $NaBH_4$ was added, causing the immediate formation of cob(I)inamide. To this solution, 0.025 mL of alkylating agent was added. The organocobinamide formed immediately. After dilution of the reaction solution with 10 mL of water, the product was extracted into a minimum volume of phenol/$CHCl_3$ and then precipitated with diethyl ether. The solid was collected by centrifugation, redissolved in a minimum volume of methanol, reprecipitated with ether, centrifuged, and dried with dry Ar. The organocobinamide was purified by chromatography on a 1.5 × 20 cm column of CM-cellulose with 10 mM pH 7.0 sodium phosphate buffer as the eluent. It was recovered from the major band by phenol extraction and precipitation as described above.

Kinetic Measurements. The rates of aerobic decomposition of the organocobalamins were determined by recording repetitive spectral scans. The decomposition of cobalamins **2–4** in neutral solution was initiated by adding a drop of a freshly prepared solution of the cobalamin in 0.1 M HCl to a cuvette containing 0.1 M pH 7.0 sodium phosphate buffer. Cobalamin **5** was dissolved in 0.1 M pH 7.0 phosphate prior to initiating the decomposition with a drop of this solution. Toward the end of each reaction, remaining organocobalamin was photolyzed to obtain the t_∞ spectrum. Sharp isosbestic points were observed, and the first-order rate constants were evaluated from the absorbance changes at the wavelength of the γ band of the Co(III)–corrin.

For the slow decomposition of cobinamide **3** and **4** in 0.10 M pH 7.0 sodium phosphate buffer, the solutions were stored in the dark. At appropriate times, aliquots were removed. These were diluted into 0.1 M HCl to accelerate the aerobic oxidation of cob(II)inamide to cob(III)inamide. After 15 min, the spectra of these solutions were recorded before and after photolysis. (The organocobinamides are stable in these acidic solutions for 15 min.)

First-order rate constants were determined from the slopes of ln $[1 - (A_t/A_{h\nu})]$ vs. time plots, where A_t is the absorbance at time t and $A_{h\nu}$ is the corresponding absorbance after photolysis, measured at the wavelength of the Co(III)–corrin γ band.

The anaerobic decomposition reactions of cobalamins **2–4** were performed in sealed-glass ampules under argon.

Aerobic Decomposition of Cobalamins 3 and 4. Cobalamin **3** (150 mg) was dissolved in 10 mL of methanol. After the solution was kept for 12 h in the dark at room temperature, decomposition to hydroxocobalamin was complete. After evaporation of the methanol, 10 mL of water was added, and the organics were extracted with diethyl ether. The products were identified by GLPC. Cobalamin **4** (250 mg) was similarly allowed to decompose in a mixture of 10 mL of methanol and 25 mL of 0.1 M pH 9.0 borate buffer. After 12 h, *O,S*-diethyl dimethylmalonate and *O,S*-diethyl methylthiomalonate were identified by TLC. A third product was purified by preparative TLC. Its 220-MHz ^1H NMR spectrum showed the presence of esters and thioesters, but no *O,S*-diethyl methylthiosuccinate. This product was not further characterized.

Reductive Cleavage Experiments. Anaerobic reduction of *O,S*-diethyl (bromomethyl)methylthiomalonate with vitamin B_{12s}/Zn was performed in a 160-mL bottle containing 805 mg of vitamin B_{12a} (0.5 mmol) in 25 mL of 1:1 C_2H_5OH/H_2O containing 2.0 g of NH_4Br. After the bottle was deaerated with Ar, 3 g of Zn dust was added. The bottle was capped with a silicone septum, and argon flushing was continued by using gas inlet and exit needles. The reduction to vitamin B_{12s} was complete within 3 min. To this solution 300 mg (1.0 mmol) of *O,S*-diethyl (bromomethyl)methylthiomalonate was injected by means of a syringe. This produced a yellow solution which reverted back to vitamin B_{12s} within 30 min of shaking. The organic products were extracted into diethyl ether after removing the ethanol by rotary evaporation. The identities and relative yields of the products were determined by GLPC and TLC. The rearranged product was separated by preparative TLC, and exclusive thioester migration was established by comparing its 220-MHz ^1H NMR spectrum with the spectra in ref 7a.

Reductive cleavage of isolated solid cobalamin **4** with $NaBH_4$ was achieved as follows: Cobalamin **4** (260 mg) was dissolved in 25 mL of a deaerated 3:1 mixture of 0.1 M sodium borate (pH 9)/methanol. To this solution was added 250 mg of $NaBH_4$. The H_2 pressure generated during the reaction of $NaBH_4$ with the solvent was relieved periodically by means of a 50-mL syringe. (To monitor the reaction, we placed an aliquot in 1 N HCl for 15 min to allow unalkylated cobalamin to oxidize. The absorption spectrum was then taken before and after photolysis.) The reaction was essentially complete after 2 h. Products were recovered as above and identified by GLPC and TLC. Cobalamin **5** was cleaved with $NaBH_4$ in 0.1 M pH 9 borate buffer by using the same procedure. In this case the reaction was complete after 16 h.

The anaerobic reductive cleavage of cobalamins **3** and 4 with zinc was studied by adding 25 mL of a deaerated 5% (w/v) solution of NH_4Br in methanol to a deaerated serum vial containing 250 mg of the organocobalamin, 1 g of zinc dust, and a magnetic stir bar. Green vitamin B_{12s} formed within 30 min.

Reductive cleavage of cobalamin **4** under aerobic conditions was performed as described in ref 7a. However, we conducted it at one fourth of the reported scale and exposed the reaction solution to air after formation of the organocobalamin.

Zinc Reduction of *O,S*-Diethyl (Bromomethyl)methylmalonate. *O,S*-Diethyl (bromomethyl)methylthiomalonate (0.1 mL) was dissolved in 25 mL of 5% (w/v) NH_4Br in methanol. To this solution 1 g of zinc dust was added, and the suspensions was stirred overnight, filtered, and evaporated. After addition of 25 mL of water, the products were extracted with ether and identified by GLPC and TLC.

Anaerobic Photolysis of Cobalamin 4. Cobalamin 4 (250 mg) was placed into a bottle of 160-mL capacity. The bottle was sealed with a silicon septum. This bottle was deaerated with argon for 2 h.

A second bottle of 160-mL capacity was filled with 100 mL of a 0.1 M solution of pH 9 borate buffer in a 3:1 water:methanol mixture. This solution was also deaerated for 2 h. Thereafter, the solution was transferred via catheter under Ar pressure into the first bottle containing 4. After an additional 15 min of flushing with Ar, the solution was photolyzed for 24 h. The solution after this time contained only 10% of residual organocobalamin. The organics were extracted into ether and identified by GLPC.

Spontaneous Decomposition Reactions of Cobalamin 5. Cobalamin **5** (350 mg) was dissolved in 25 mL of a deaerated 0.1 M pH 7 phosphate buffer solution in a deaerated serum vial. The solution was allowed to sit for 42 h. Products were extracted with CH_2Cl_2. Analysis by GLPC showed the presence of diethyl methylmalonate; diethyl succinate was not detected. This result was confirmed by TLC. In addition, a major product was observed which did not migrate from the origin. The 220-MHz ^1H NMR spectrum of this apparently polymeric product or product mixture revealed traces of diethyl methylenemalonate as well as diethyl methylmalonate. The major peaks were similar to those of the polymer

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(25) Sumrell, G.; Zief, M.; Huber, E. J.; Ham, G. E.; Schram, C. H. *J. Am. Chem. Soc.* **1959**, *81*, 4313.

derived from adding authentic diethyl methylenemalonate into pH 7 phosphate buffer.

The spontaneous aerobic decomposition of **5** was studied by dissolving 300 mg of the cobalamin in 25 mL of pH 7 phosphate buffer. The resulting solution sat for 42 h at room temperature in the dark, and products were analyzed as above. The major product did not migrate on TLC and had an ^1H NMR spectrum similar to that of the methylenemalonate polymer obtained in the experiments described above.

Vitamin B_{12s} Trapping Experiments. Cobalamin **5** (37 mg) in a deaerated serum vial was dissolved in 20 mL of a deaerated 3:2 methanol/0.1 M/pH 7 phosphate buffer solution containing 0.4 mL of ethyl bromide. After 42 h, the optical absorption spectrum of an aliquot before and after photolysis revealed 90% ethylcobalamin. The identity of this cobalamin was verified after isolating it by phenol extraction and precipitation with ether. Photolysis of a sample produced ethylene. It also exhibited the same R_f as authentic ethylcobalamin on TLC on cellulose, with 1-butanol:ethanol:water (10:3:7, v/v/v) as the eluent.

Reactions of Cob(I)alamin with Bromosuccinate and Fumarate Esters. In typical experiments, a solution of 311 mg of vitamin B_{12a} (82.9%, 184 μmol) in 10 mL of 5% (w/v) NH_4Br in methanol in a 15-mL centrifuge tube was deaerated with argon. After 300 mg of Zn dust was added, the tube was sealed with a rubber septum stopper. Flushing with Ar continued for 5 min, during which vitamin B_{12s} formation was complete. After centrifugation to pellet the zinc, the supernatant was transferred via catheter under Ar pressure into a deaerated serum vial containing 53 mg (210 μmol) of diethyl bromosuccinate in 6 mL of 5% NH_4Br in methanol. The solution immediately became brown and spectral analysis of an aliquot indicated that vitamin B_{12r} had been formed.

After 5 min, the vial was opened and the solvent removed by rotary evaporation. The residue was separated between water and ether. The ether was dried with $MgSO_4$ and removed by rotary evaporation. A ^1H NMR spectrum of the residue in CCl_4 was taken, revealing a 1:1 mixture of diethyl succinate and diethyl bromosuccinate. A control with no vitamin B_{12} left diethyl bromosuccinate unchanged.

In other experiments, diethyl fumarate and *S,S*-diethyl dithiofumarate were reacted with hydridocobalamin in 1:1 C_2H_5OH:CH_3COOH, and separately, *S,S*-diethyl dithiofumarate was reacted with vitamin B_{12s} generated in 5% methanolic NH_4Br. The zinc used to reduce the cobalt must always be removed to prevent its reaction with the alkylating agent. Product mixtures were analyzed by NMR as above. In addition, products from *S,S*-diethyl dithiofumarate were analyzed for *S,S*-diethyl methyldithiomalonate by TLC on silica gel (0.25 mm), using benzene:hexanes (2:1) as the solvent and running the solvent 2-3 times. No rearranged product was detected.

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Registry No. 1, 71721-47-6; cobalamin **2**, 80662-49-3; cobalamin **3**, 80679-15-8; cobalamin **4**, 62430-52-8; cobalamin **5**, 80662-50-6; neopentylcobinamide, 80679-16-9; cobinamide **3**, 80662-51-7; cobinamide **4**, 80662-52-8; vitamin B_{12s}, 18534-66-2; vitamin B_{12g}, 13422-51-0; diaquocobinamide, 80662-53-9; methylmalonyl-CoA mutase, 9023-90-9; diethyl methylmalonate, 609-08-5; *O,S*-diethyl methylthiomalonate, 80658-34-0; *O,S*-diethyl dimethylthiomalonate, 62442-85-7; *O,S*-diethyl 2-methyl-4-thiosuccinate, 62442-84-6; diethyl methylsuccinate, 4676-51-1; *O,S*-diethyl (bromomethyl)methylthiomalonate, 62442-83-5; diethyl bromosuccinate, 763-51-9; diethyl fumarate, 623-91-6; *S,S*-diethyl dithiofumarate, 62674-35-5.

Twisted Carbon–Carbon Double Bonds. Crystal and Molecular Structure of 4,5-Di-*tert*-butyl-1,1,2,2-tetrafluoro-1,2-disilacyclohexa-3,5-diene

Tzong-Hsiung Hseu,* Yun Chi, and Chao-Shiuan Liu*

Contribution from the Institute of Molecular Biology and Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan, Republic of China. *Received June 4, 1981*

Abstract: 4,5-Di-*tert*-butyl-1,1,2,2-tetrafluoro-1,2-disilacyclohexa-3,5-diene, $C_{12}H_{20}Si_2F_4$, crystallizes in a monoclinic system with space group $P2_1/c$, a = 12.584 (5) Å, b = 16.843 (7) Å, c = 15.293 (4) Å, and β = 69.70 (3)°. The density calculated is 1.295 g cm^{-3} with Z = 8. Intensity data were obtained on a single-crystal diffractometer with Cu Kα radiation. The crystal structure was solved by direct methods and refined by full-matrix least squares to R = 0.083 on F for 1923 independent reflections. The two crystallographic independent molecules are related approximately by a pseudotwofold rotation symmetry. Both molecules have similar dimensions and adopt a twisted-boat conformation which deviates somewhat from C_2 symmetry. The bonds connecting the two *tert*-butyl groups are long, averaging 1.54 (2) Å, as compared to the normal C(sp^2)–C(sp^2) single bond length of 1.48 Å. The averaged Si–Si bond length and Si–Si–C bond angles are 2.331 (7) Å and 95.5 (5)°, respectively. The carbon–carbon double bonds are all twisted and can be understood on the basis of the polarized zwitterionic transition state with the ring strain acting as the steric source and *tert*-butyl and SiF_2 parts as the donor and acceptor groups. The averaged C=C bond lengths and twisted angles are 1.345 Å for a twist of 24° and 1.388 Å for a 27° twist. Both ^{13}C NMR and UV spectra are consistent with this picture.

The twisted carbon–carbon double bond has been a subject of continuous theoretical interest for many years.[1] One recent interest particularly lies on the proposed "sudden polarization" effect of the twisted excited state of olefins and its possible role in vision.[2] At ground state, efforts have been taken in the studies of ethylenes with strong donor groups on one carbon atom and strong acceptors on the other ("push–pull" effect).[3,4] When sufficient steric interactions between these groups exist, these ethylenes are permanently twisted around the carbon–carbon double bonds.[3-5]

One of the most prominent steric interactions in the 1,3-diene system is the case of 2,3-di-*tert*-butyl-1,3-butadiene.[6,7] The

* T.-H.H., Institute of Molecular Biology. Y.C. and C.-S.L., Department of Chemistry.

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