might also give rise to stable transition metal–olefin π complexes.

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Formylmethylcobalamin

Sir:

It has been postulated that formylmethylcobalamin (**1**) is an intermediate in the enzymic conversion of ethylene glycol to acetaldehyde in a coenzyme-B_{12} dependent reaction.[1,2] The synthesis of this compound has recently been reported as well as some of its chemical properties.[3] We have also synthesized this compound and have found significant differences in its chemical properties from those reported. Since this compound has been proposed as an intermediate in a reaction of considerable interest, we now report the properties of the compound we have observed.

Formylmethylcobalamin was synthesized in four different ways, which are outlined in Figure 1. Each synthetic scheme resulted in the same product. The methods of synthesis establish the structure of the compound as formylmethylcobalamin. The product was characterized through its uv and visible absorption spectrum[4] and the R_f value in several chromatographic systems.[5] The rates of acid hydrolysis of the compound prepared by any of the above routes were identical (see below). Acetaldehyde and hydroxocobalamin were quantitatively identified after acid hydrolysis (acetaldehyde by glc and hydroxocobalamin by its optical spectrum). The same products were qualitatively identified after aerobic and anaerobic photolysis.[6]

The properties of formylmethylcobalamin, which we have observed, differ from those reported previously in two respects. It was reported that the nmr spectrum of formylmethylcobalamin showed a triplet at 9.01 ppm (solvent not stated).[3] The compound which we have shows a broad triplet at 8.22 ppm[7] (in D_2O).

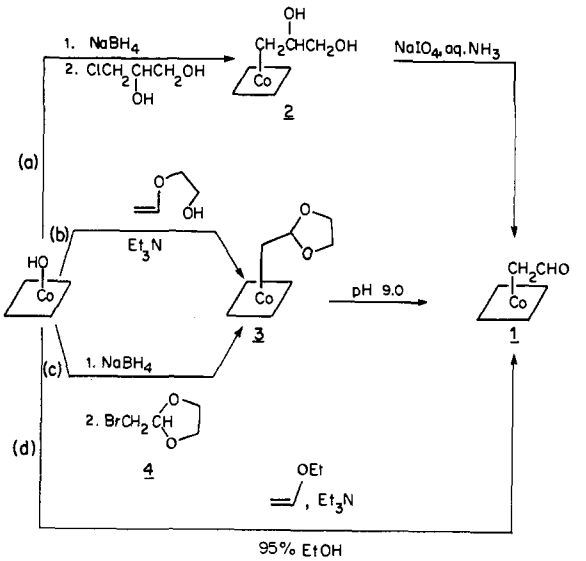


Figure 1. (a) Hydroxocobalamin was reduced by $NaBH_4$ to B_{12}(s) and then alkylated with 3-chloro-1,2-propanediol. The product was desalted (D. Dolphin, *Methods Enyzmol.*, **18c**, 34 (1971)) and applied to silica gel plates, which were developed in 1-propanol:water:concentrated ammonia (100:99:1). The resulting 2,3-dihydroxypropylcobalamin (**2**) ran as a broad band with R_f = 0.3–0.5 and was eluted and oxidized with meta periodate in aqueous ammonia.[6] (The oxidation was followed by measuring release of formaldehyde by a modification of the method described in E. R. Frisell, L. A. Meech, and C. G. MacKenzie, *J. Biol. Chem.*, **207**, 709 (1954).) The formylmethylcobalamin was separated from hydroxocobalamin (also a product in the oxidation reaction) by passing the mixture through a column of Bio-Gel P-2 (200–400 mesh) in aqueous ammonia. (b, c) Preparation of 1,3-dioxa-2-cyclopentylmethylcobalamin (**3**) from hydroxocobalamin and 2-hydroxyethyl vinyl ether is described in the preceding paper. See R. B. Silverman and D. Dolphin, *J. Amer. Chem. Soc.*, **96**, 7094 (1974). The same product is obtained from the bromoacetal **4** and B_{12}(s). Partial hydrolysis of the acetal to formylmethylcobalamin occurred after 48 hr at pH 9.0 in 0.025 *M* Borax–0.1 *N* HCl buffer (R. C. Weast, Ed., "Handbook of Chemistry and Physics," 46th ed., The Chemical Rubber Co., Cleveland, Ohio, 1965, p D-73). The product was separated from hydroxocobalamin and the starting acetal by chromatography on cellulose plates. (d) Hydroxocobalamin and ethyl vinyl ether were allowed to stand at room temperature for 6 days in 95% ethanol. On chromatography,[5] formylmethylcobalamin and 2,2-diethoxyethlcobalamin were resolved in the ratio of about 4 to 1.

The broadening may be due to the interaction with the diastereotopic methylene protons. The diastereotopic nature of the methylene protons of cobalamin has previously been reported.[8,9]

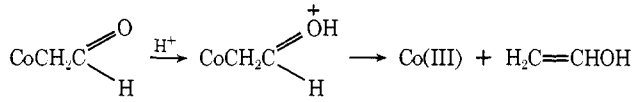
It was also reported that formylmethylcobalamin is acid stable,[3] and an optical spectrum of this compound at pH 5.8 was shown. We find that formylmethylcobalamin is acid sensitive. The rate of decomposition at various pH values for formylmethylcobalamin synthesized through two different routes is shown in Table I. Note that $t_{1/2}$ at pH 5.8 is 3 min.[10] The rate law for decomposition is $d[\text{formylmethylcobalamin}]/dt = -k[\text{formylmethylcobalamin}][H_3O^+]$, where k = 2040 M^{-1} sec^{-1}.

(1) R. H. Abeles, *Advan. Chem. Ser.*, No. 100, 346 (1971).
(2) T. J. Carty, B. M. Babior, and R. H. Abeles, *J. Biol. Chem.*, **246**, 6313 (1971).
(3) G. N. Schrauzer, W. J. Michaely, and R. J. Holland, *J. Amer. Chem. Soc.*, **95**, 2024 (1973).
(4) The spectrum was typical of an alkylcobalamin, $\lambda_{nm}^{H_2O}$ ($\epsilon \times 10^{-3}$): 262 (26.3), 278 (23.3), 288 sh (20.0), 334 (15.1), 370 (13.9), 430 (5.42), 496 sh (6.54), 526 (8.37), 550 sh (7.12).
(5) Formylmethylcobalamin has an R_f value of 0.51 on Brinkmann cellulose plates developed in 1-butanol:ethanol:water (10:3:7) containing 0.5% concentrated aqueous ammonia. Products from all synthetic routes also cochromatographed when the plates were developed in *n*-butyl alcohol:isopropyl alcohol:water (7:6:7) containing 0.5% concentrated aqueous ammonia (R_f = 0.54) or *sec*-butyl alcohol:methanol:water (55:15:30) containing 0.5% concentrated aqueous ammonia (R_f = 0.45).
(6) T. J. Carty, Ph.D. Thesis, Brandeis University, 1973.
(7) The methine proton of aliphatic aldehydes normally appears in the region δ 9–10. A value of 8.22 for formylmethylcobalamin is consistent with the shielding, and consequent upfield shift, experienced by protons on the β-carbon of an alkylcobalamin.
(8) J. D. Brodie and M. Poe, *Biochemistry*, **11**, 2534 (1972).
(9) J. D. Brodie and M. Poe, *Biochemistry*, **10**, 914 (1971).
(10) Acetate buffers, prepared by mixing 0.1 *M* solutions of sodium acetate and acetic acid, were used for runs with pH <6; phosphate buffers, prepared by mixing 0.1 *M* solutions of mono- and dibasic sodium phosphate, were used for runs with pH >6.

Table I. Acid Decomposition of Formylmethylcobalamin

pH	$t_{1/2}$, min: Prepared by oxidation of 2,3-dihydroxypropylcobalamin	$t_{1/2}$, min: Prepared by hydrolysis of 1,3-dioxa-2-cyclopentylmethylcobalamin
5.3	1.1	1.1
5.8		3.1
6.2	6.7	6.7
6.5	12.2	12.1
6.8	21.9	21.6

Hydroxyethylcobalamin and hydroxyethylcobinamide are acid sensitive and decompose *via* cobalt–carbon bond cleavage. The rate law for the decomposition of hydroxyethylcobinamide is d[hydroxyethylcobinamide] = $-k_2[H^+]$[hydroxyethylcobinamide] = $k_2[H^+]$[hydroxyethylcobinamide] where k_2 = 0.0047 M^{-1} sec^{-1}.[11] We therefore expect formylmethylcobalamin to be extremely acid sensitive and propose the following scheme for its acid decomposition.



The heterolytic decomposition of formylmethylcobalamin upon photolysis has recently been cited as evidence against its participation in the enzymic mechanism.[3] Since interaction of the enzyme with the cobalamin will clearly modify its chemistry, we feel conclusions drawn about enzymic mechanisms from unrelated photochemical evidence are invalid.

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(11) P. Dunne, Ph.D. Thesis, Brandeis University, 1970.
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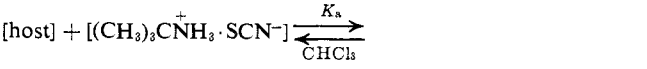
Structural Parameters That Control Association Constants between Polyether Host and Alkylammonium Guest Compounds[1]

Sir:

Selective association between organic host and guest compounds to form highly structured molecular complexes of ground or transition states is a phenomenon central to nature's enzymatic, regulatory, and transport systems. Systematic study of the structural features of organic molecular complexation in solution not involving proteins largely has been limited to the three cyclodextrins as hosts dissolved mainly in aqueous media. The structures of the guest molecules have been varied widely.[2] Chiral recognition by design of molecular complexes has demonstrated that a high degree of molecular organization is possible by arranging complementary binding sites and steric barriers in host and guest.[3] This paper reports how association constants between *tert*-butylammonium thiocyanate and multiheteromacrocycles in chloroform vary with structural parameters of the host.

Table I reports the association constants for 28 multiheteromacrocycles and two open-chain model compounds as hosts and *tert*-butylammonium thiocyanate as guest in chloroform (eq 1) at 24 and 0°.[4]



$$[\text{host}] + [(CH_3)_3\overset{+}{C}NH_3 \cdot SCN^-] \underset{CHCl_3}{\overset{K_a}{\rightleftharpoons}} [(CH_3)_3\overset{+}{C}NH_3 \cdot \text{host} \cdot SCN^-] \quad (1)$$

The interesting correlations between structure and complexing power (Table I) are as follows. (1) Compound **18**, whose aryl oxygens are distant (para) from one another, has a K_a whose value is a factor of $>3.5 \times 10^3$ lower than K_a of isomer **5**, whose aryl oxygens are ortho. In a complex of **18** and RNH_3^+, a maximum of three oxygens at a time can be used in binding, whereas in that of **5**, all six can be involved in complexation.[5] (2) Compound **2** (18-crown-6) possesses a binding constant $>10^4$ higher than its conformationally flexible open-chain counterpart, **1**, and cyclic binaphthyl compound **29** possesses a constant ~10 times that of noncyclic binaphthyl compound, **28**. The conformations of the complexed and noncomplexed states of the cycles are more similar than those of the open-chain compounds. The rigid binaphthyl unit in the middle of the chain reduces the differences between the cyclic and the noncyclic host. (3) Substitution by a methylene of an oxygen of **2** as in **3** reduced the constant by a factor of

(1) This work was supported by a grant from the National Science Foundation, GP33533X, and by the U. S. Public Heaith Service, Research Grant No. GM12640-10 from the Department of Health, Education and Welfare.

(2) (a) D. W. Griffiths and M. L. Bender, *Advan. Catal. Relat. Subj.*, **23**, 209 (1973); (b) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, N. Y., 1974, Chapter 11.

(3) (a) D. J. Cram and J. M. Cram, *Science*, **183**, 803 (1974); (b) R. C. Helgeson, J. M. Timko, P. Moreau, S. C. Peacock, J. M. Mayer, and D. J. Cram, *J. Amer. Chem. Soc.*, **96**, 0000 (1974).

(4) A 0.14 *M* solution of host in $CDCl_3$ (0.6 ml) was shaken at 24 or 0° with 1.6 ml of 0.1 *M* $(CH_3)_3CNH_3^+SCN^-$ in D_2O (scale A), with 0.6 ml of 0.4 *M* salt (scale B), or with 0.3 ml of 1.0 *M* salt (scale C). With 100-MHz pmr spectra, the relative concentrations of guest (CH_3 protons) to host (all protons) in $CDCl_3$ were measured (±2%). The host in D_2O was ≲0.5% of the total used except for **2** (Table I, footnote *c*). The absolute amounts at equilibrium of salt extractable at 24 and 0° were determined by large scale experiments in the absence of host at initial guest concentrations of scales A, B, and C. Values of *K* were calculated from eq I for each scale in which $[BX]_{D_2O}$ and $[BX]_{CDCl_3}$ were equi-

$$K = \frac{[BX]_{D_2O}{}^2 R}{[BX]_{CDCl_3}(1 - R)\,[[BX]_i - [H]_i R(V_{CDCl_3}/V_{D_2O})]^2} \quad (I)$$

librium concentrations of salt in the absence of host, *R* is the ratio of concentrations of guest to host in $CDCl_3$ at equilibrium, $[BX]_i$ is the initial salt concentration in D_2O, $[H]_i$ is the initial host concentration in $CDCl_3$, and V_{CDCl_3} and V_{D_2O} are the volumes of $CDCl_3$ and D_2O. Scales A and B were corrected to scale C by multiplying *K* values for scales A and B by 1.5 to give K_a values. This factor (±20%) represents an average of the factors by which the *K*'s of several hosts common to scales A and C or B and C differed.

(5) Corey–Pauling–Koltun molecular models.