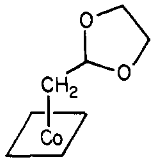
On the Mechanism of Hydrolysis of β-Acetal Cobalamins

Sir:

It has been reported[1,2] that both 1,3-dioxa-2-cyclopentylmethylcobalamin (**1**) and 2,2-diethoxyethylcobalamin (**2**) hydrolyze by direct and exclusive cobalt-carbon bond heterolysis to give hydroxocobalamin (B_{12b}) with no intermediate formation of formylmethylcobalamin (**3**). Despite this we have found[3] that the hydrolysis, at pH 9.0, of **1** affords a convenient preparation of formylmethylcobalamin (**3**). In order to further clarify the situation we have investigated both the kinetics and mechanism of the hydrolysis of the acetals **1** and **2**.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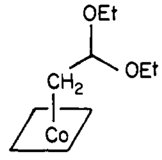
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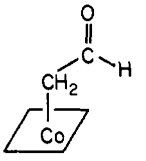
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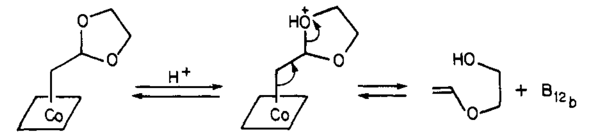
Semilogarithmic plots of $OD_\infty - OD_t$ vs. time[4] as a function of pH for the decomposition of **1**, prepared by the oxidative addition of bromoethylidene ethylene glycol with B_{12s}, and by the addition of 2-hydroxyethyl vinyl ether to B_{12b},[5] were linear. The half-life for decomposition at each pH value was nearly identical with those found for formylmethylcobalamin,[3] the second-order rate constant being 2070 M^{-1} sec^{-1}.

These data suggest two possible mechanisms: hydrolysis of the acetal **1** to the aldehyde **3** in a fast step, followed by a rate-determining cobalt-carbon bond cleavage, or direct cobalt-carbon bond cleavage to B_{12b} and hydroxyethyl vinyl ether (Scheme I). This latter mechanism would, however, require that the reaction occurs with a rate constant which is coincidentally the same as that for the decomposition of formylmethylcobalamin.

Scheme I



If the former two-step mechanism is operative then an accumulation of formylmethylcobalamin must occur. When the hydrolysis of **1** was followed by TLC[6] at pH 9.0, a steady state concentration of about 25% formylmethylcobalamin was observed; at pH 7.0 only 10% of the aldehyde was seen. As the pH is increased decomposition of formylmethylcobalamin becomes slower, and thus an increase in its concentration, with increasing pH, is observed. When, however, the acetal **1** was decomposed at pH 6.5 or 6.8 in phosphate buffer, and the products analyzed by gas chromatography,[7] 2-hydroxyethyl vinyl ether was also detected. This suggests that at lower pH's both mechanisms may be operative.

The same kinetic study was carried out with 2,2-diethoxyethylcobalamin (**2**), when it was found that this cobalamin decomposed more rapidly than the cyclic acetal. Semilogarithmic plots of $OD_\infty - OD_t$ vs. time were linear for the first half-life, and then showed an upward deviation (i.e., the reaction proceeded more slowly). When the kinetics were followed for more than ten half-lives, the initial rate converted to the slower first-order kinetics observed for the decomposition of formylmethylcobalamin (Figure 1).

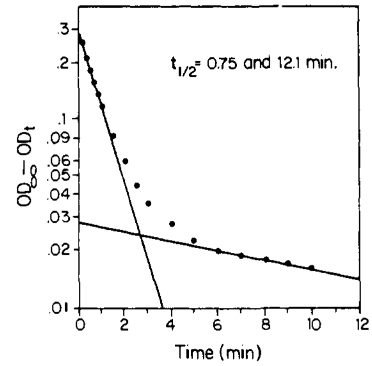


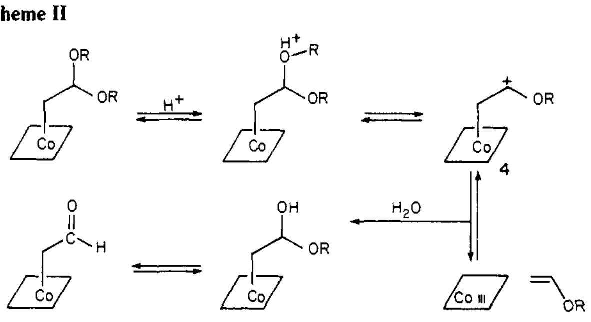
Figure 1. Semilogarithmic plot of $OD_\infty - OD_t$ vs. time for the decomposition of 2,2-diethoxyethylcobalamin in phosphate buffer at pH 6.5 and 25°. Decomposition was followed by observing the increase in optical density at 350 nm.

Thus it appears that the acyclic acetal also decomposes by two different pathways. The second-order rate constant is 50,000 M^{-1} sec^{-1} for the initial rapid decomposition, and 2040 M^{-1} sec^{-1} for the slower decomposition. When the hydrolysis was followed by TLC[6] only very small amounts of formylmethylcobalamin were observed. Since hydrolysis of formylmethylcobalamin is much slower than the direct cleavage of the acyclic acetal, the overall amount of observed formylmethylcobalamin indicates that only a small amount is formed.

The rapid decomposition of the acyclic acetal **2** compared to that of the cyclic acetal **1** arises from the differing amounts of direct cobalt-carbon bond cleavage. Fife and Jao[8] found that the diethyl acetal of benzaldehyde hydrolyzes 28 times faster than the corresponding 1,3-dioxalane. Similarly the acyclic cobalamin acetal hydrolyzes 25 times faster than the cyclic counterpart. Here, as with the benzaldehyde acetals, the less favorable entropy of activation for the cyclic acetal presumably accounts for the difference in rates. That direct cobalt-carbon fission occurs with 2,2-diethoxyethylcobalamin was shown by the detection[9] of ethyl vinyl ether as a product of its decomposition.

Thus both cobalamin acetals **1** and **2** decompose by hydrolysis to formylmethylcobalamin, followed by cobalt-carbon bond cleavage to B_{12b} and acetaldehyde[3] and by direct cobalt-carbon bond fission. These two processes can be envisioned as proceeding via the common carbonium ion intermediate **4** (Scheme II).

Scheme II



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References and Notes

(1) G. N. Schrauzer, W. J. Michaely, and R. J. Holland, *J. Am. Chem. Soc.*, **95**, 2024 (1973).
(2) W. J. Michaely and G. N. Schrauzer, *J. Am. Chem. Soc.*, **95**, 5771 (1973).

(3) R. B. Silverman, D. Dolphin, T. J. Carty, E. K. Krodel, and R. H. Abeles, *J. Am. Chem. Soc.*, **96**, 7096 (1974).

(4) The kinetics of decomposition were followed by observing the increase in the optical absorption at 350 nm, the γ-band of aquocobalamin, at pH 5.3, 5.8, 6.2, 6.5, and 6.8. In each case, an aqueous solution of the acetal cobalamin was diluted tenfold in 0.1 *M* buffer; the end-point was determined as the OD given by the solution at each pH after photolysis.

(5) R. B. Silverman and D. Dolphin, *J. Am. Chem. Soc.*, **95**, 1686 (1973).

(6) Brinkman cellulose plates (Celplate-22 without indicator) eluted with 1-butanol:ethanol:water (10:3:7) containing 0.5% concentrated aqueous NH_3. R_f values for cyclic acetal, diethyl acetal, aldehyde, and B_{12b} are 0.56, 0.62, 0.51, and 0.22, respectively.

(7) Hewlett-Packard 7620A research chromatograph with flame ionization detector; 6 ft 5% Carbowax 20M; 80–100W; 65°; 33 cm/min helium flow.

(8) T. H. Fife and L. K. Jao, *J. Org. Chem.*, **30**, 1492 (1965).

(9) Hewlett-Packard 5750 research gas chromatograph; thermal conductivity detector; 20 ft 10% β,β-oxydipropionitrile; 80–100 WHP; 50°; 41 cm/min helium flow.

(10) National Institutes of Health Predoctoral Trainee.

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Molecular Heteronuclear Chelates. Mixed Metal Chelates of *N,N*^{1}-Bis(1-phenyl-1,3,5-hexanetrione)ethylenediamine. Preparation and Structure

Sir:

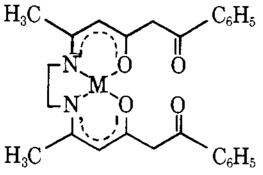
Molecular complexes containing two or more different metal ions in predictable environments are not well known. Such molecules are of interest in the areas of metalloenzymes, homogeneous catalysis, and magnetic exchange interactions. We wish to report the general synthesis and detailed characterization of a series of such complexes. The synthesis of these *pure* mixed polynuclear chelates, hereafter referred to as heteronuclear chelates, employs binucleating ligands in which the coordination sites have different donor atoms, thus associating coordination selectivity with each site. The metals are introduced stepwise with characterization of the intermediate mononuclear and the final heteronuclear products.

The characterization outlined below includes spectral and magnetic analyses of the compounds prepared as well as three-dimensional X-ray diffraction studies of several members of the series. Correlation of the magnetic and structural results unambiguously establishes the final products as pure heteronuclear complexes.

The new class of ligands employed are the Schiff-bases formed by the condensation of a diamine and a 1,3,5-triketone. These ligands are potentially tetraanionic and by analogy to the chelates of 1,3,5-triketonates can be expected to chelate two divalent metal ions giving a neutral binuclear molecule.[1-5] The Schiff base prepared from 1-phenyl-1,3,5-hexantrione[6] and ethylenediamine, hereafter abbreviated $H_4(BAA)_2en$,[7] is readily prepared in 95% yield by mixing stoichiometric quantities of the reactants in boiling absolute methanol. On cooling to 0°, yellow crystals form which were recrystallized from methanol (mp 171°). Depending upon reaction conditions, $H_4(BAA)_2en$ may be complexed to one or two metal ions. Several examples of each type are discussed below. The analytical results for all the compounds described herein are in excellent agreement with the indicated formulations.

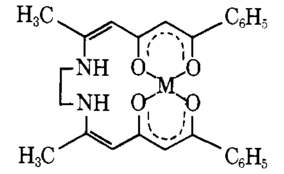
Other related homonuclear chelates have been reported that contain binucleating macrocyclic ligands. Fenton and Gayda[8] reported the preparation of a binuclear complex of the macrocyclic ligand resulting from the condensation of 2,4,6-heptanetrione and ethylenediamine. In addition, Ueno and coworkers[9] and Pilkington and Robson[10] have synthesized several homonuclear complexes using a macrocyclic ligand based on 2,6-disubstituted phenol. There is also one report of a heteronuclear complex of a related ligand in which the metals are Cu(II) and Ni(II).[11] Characterization of this molecule was on the basis of spectral and magnetic data. No structural information is available for any of these chelates.

A number of mononuclear chelates of $H_4(BAA)_2en$ were prepared by allowing 1:1 molar ratios of metal acetates or fresh hydroxides and the ligand to react in H_2O–acetone mixtures. In each case, two isomers (I and II) may result.



I

$M[H_2(BAA)_2en]\text{-}N_2O_2$



II

$M[H_2(BAA)_2en]\text{-}O_2O_2$

The donor atoms are shown after the general formula as a means of distinguishing between the positional isomers. It is found that, for certain metals, one isomer (I or II) is uniquely produced.

A. $M[H_2(BAA)_2en]\text{-}N_2O_2$ Isomer. The mononuclear Ni(II) chelate is an example of isomer I. This complex is readily isolated as red crystals (mp 174°) which are soluble in most organic solvents and insoluble in water. More specifically, the product is extremely soluble in $CHCl_3$ and C_6H_6, but considerably less soluble in acetone and alcohols. Although no structural determination was undertaken, the following spectral and magnetic arguments are sufficient to establish that I is produced as the only product.

First, the infrared spectrum of $Ni[H_2(BAA)_2en]$ contains an intense unchelated carbonyl band at 1700 cm^{-1} which would be absent if the coordination were O_2O_2. Second, the visible spectrum is essentially identical with that of $Ni(acac)_2en$ where the coordination geometry is the same as in I. Third, the product is rigorously diamagnetic ($\chi_g(\text{obsd}) = -266 \times 10^{-6}$, $\chi_g(\text{calcd}) = -246 \times 10^{-6}$ cgs) and, therefore, the Ni(II) is square planar. $Ni(acac)_2en$, a model for isomer I, is also diamagnetic and square planar.

Although the spectral data cannot be used to assess the purity with respect to positional isomerism, magnetic results may be used in this way. If the Ni(II) were coordinated at the O_2O_2 site, II, its environment would be similar to that found in the bis(benzoylacetonate)nickel(II) chelate which achieves a coordination number of six by binding two adduct ligands such as water or by oligomerization.[12] In either of these environments Ni(II) is paramagnetic with $\chi_m \simeq 5000 \times 10^{-6}$ cgs at 300°K and $15,000 \times 10^{-6}$ cgs at 77°K. Thus, very small amounts of isomer II, as an impurity in I, would be easily detected by susceptibility measurements. Inasmuch as the calculated and observed diamagnetism of $Ni[H_2(BAA)_2en]$ are in such good agreement, it is possible to state that isomer I is the only product.

B. $M[H_2(BAA)_2en]\text{-}O_2O_2$ Isomer. Two well-characterized mononuclear chelates with structure II have been prepared in which M = Cu(II) and VO(II). Since spectral and magnetic characterizations are not as definitive as in the Ni(II) complex, single-crystal X-ray determinations were carried