trimethylbenzaldehyde and benzaldehyde in THF at 0 °C showed no significant difference in their reactivity (product ratio, **3a**/**3d** = 6/4). Thus, the formation of the butyl adduct from **1d** is the result of retardation of the concerted insertion by the bulky substituent. Unless primary alkoxides have sterically demanding substituents, we can deduce that they are inserted through a concerted mechanism.

The scope and limitation of the present mechanism must be defined not only by the steric requirements between alkoxides and the carbenoid[9] but also in terms of hydride transfer reactivity of alkoxides.

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Supplementary Material Available: ^{1}H NMR, IR, mass spectra, and high resolution mass spectral data of **3a–d**, **4**, **7–9**, **11**, 1-(2,4,6-trimethylphenyl)pentanol, and 1-(*p*-chlorophenyl)-3-methyl-2-butenol (4 pages). Ordering information is given on any current masthead page.

(9) The importance of the sterically demanding character of alkylidenecarbene[10] is inferred from the comparison of the present result with the stereospecific insertion by vinylidene carbene into the α-C–H bond of a secondary alkoxide.[3c]

(10) Apeloig, Y.; Karni, M.; Stang, P. J.; Fox, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 4781.

Studies of Enzyme Stereochemistry. Elucidation of the Stereochemistry of the Reaction Catalyzed by Cob(I)alamin Adenosyltransferase

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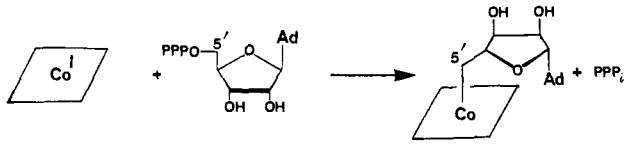
The coenzyme form of vitamin B_{12} is synthesized in living systems by the reaction of ATP with reduced vitamin B_{12} (B_{12s}) under the influence of the enzyme cob(I)alamin adenosyltransferase. This enzyme is present in homogenates of liver and kidney[1] and in extracts of Hela cells grown in tissue culture.[2] Partially purified forms of the enzyme are available from *Clostridium tetanomorphum*[3-5] and *Propionibacterium shermanii*.[6] In the reaction catalyzed by the *Clostridium* enzyme, the formation of a carbon–cobalt bond between C-5′ of ATP and B_{12s} is accompanied by the release of inorganic triphosphate (eq 1).



Evidence has also been obtained which suggests that the reaction catalyzed by the *Clostridium* enzyme may involve formation of an adenosyl-enzyme intermediate.[7] The importance of coenzyme B_{12} and the unusual mechanistic features of *Clostridium* B_{12s}-adenosyltransferase have led us to carry out a stereochemical analysis whose results are reported here.

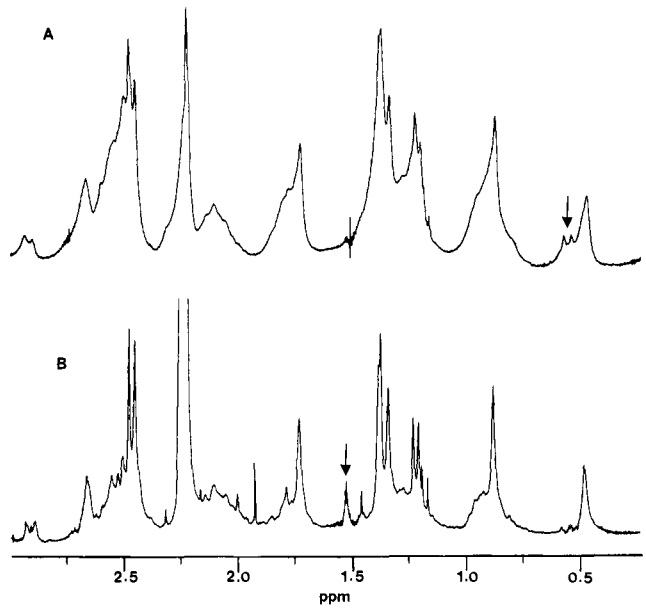


Figure 1. (A) Adenosylcobalamin derived from 5′(*R*)-(5′-$^2H_1$)ATP. (B) Adenosylcobalamin derived from 5′(*S*)-(5′-$^2H_1$)ATP. The NMR spectra were taken at 270 MHz in D_2O.

Clostridium tetanomorphum (ATCC 3606) was grown anaerobically according to the procedure of Barker et al.[8] Cob(I)-alamin adenosyltransferase was isolated from lyophilized *C. tetanomorphum* cells by a modification of published methods.[3,4] The partially purified enzyme was assayed by HPLC[9] and all manipulations were carried out in dim red light.

Incubation of cob(I)alamin adenosyltransferase with 5′(*R*)-(5′-$^2H_1$)ATP and 5′(*S*)-(5′-$^2H_1$)ATP yielded two samples of chirally deuterated coenzyme B_{12}, which were isolated by preparative reverse-phase HPLC on a C_{18} 4.6 × 250 mm column. The 270-MHz ^{1}H NMR spectra of these enzymatically derived samples of (5′-$^2H_1$)coenzyme B_{12} are shown in Figure 1. The resonance positions of the two diastereotopic hydrogen atoms at C-5′ of coenzyme B_{12} have been assigned: The 5′ *pro-R* hydrogen appears as a triplet at ca. 0.59 ppm and the 5′ *pro-S* hydrogen appears as a doublet at ca. 1.54 ppm.[10,11] An examination of the spectra shown in Figure 1 reveals the 5′(*R*)-(5′-$^2H_1$)ATP yields coenzyme B_{12} which shows a doublet at ca. 0.57 ppm with the trace of a singlet at ca. 1.54 ppm. On the other hand, the NMR spectrum of coenzyme B_{12} derived from 5′(*S*)-(5′-$^2H_1$)ATP exhibits a singlet at ca. 1.54 ppm and traces of a doublet at ca. 0.57 ppm.[12] Together, these two spectra clearly demonstrate that the formation of coenzyme B_{12} from ATP is a stereospecific process which proceeds with overall *inversion* of configuration at C-5′ of the adenosyl moiety. The same stereochemical result has been observed with the only other known adenosyltransferase, L-methionine *S*-adenosyltransferase.[13]

The formation of coenzyme B_{12} from ATP with overall inversion of configuration at C-5′ of the nucleoside strongly suggests that

(1) Pawelkiewicz, J.; Gorna, M.; Fenrych, W.; Magas, S. *Ann. N. Y. Acad. Sci.* **1964**, *112*, 641.
(2) Kerwar, S. S.; Spears, B. M.; Weissbach, H. *Arch. Biochem. Biophys.* **1971**, *142*, 121.
(3) Vitols, E.; Walker, G. A.; Huennekens, F. M. *J. Biol. Chem.* **1966**, *241*, 1455.
(4) Walker, G. A.; Murphy, S.; Huennekens, F. M. *Arch. Biochem. Biophys.* **1969**, *134*, 95.
(5) Peterkofsky, A.; Weissbach, H. *J. Biol. Chem.* **1963**, *238*, 1491.
(6) Brady, R. O.; Castanera, E. G.; Barker, H. A. *J. Biol. Chem.* **1962**, *237*, 2325.
(7) Peterkofsky, A. *Biochem. Biophys. Res. Commun.* **1966**, *24*, 310.
(8) Barker, H. A.; Smyth, R. D.; Weissbach, H.; Munch-Peterson, A.; Tooley, J. I.; Ladd, J. N.; Volcani, B. E.; Wilson, R. M. *J. Biol. Chem.* **1960**, *235*, 181.
(9) Frenkel, E. P.; Kitchens, R. L.; Prough, R. *J. Chromatogr.* **1979**, *174*, 391.
(10) Cheung, A.; Parry, R.; Abeles, R. H. *J. Am. Chem. Soc.* **1980**, *102*, 384.
(11) Gaudemer, A.; Zylber, J.; Zylber, N.; Baran-Marszac, M.; Hull, W. E.; Fountoulakis, M.; König, A.; Wölfle, K.; Rétey, J. *Eur. J. Biochem.* **1981**, *119*, 279.
(12) The residual signals at 1.54 and 0.57 ppm in the spectra of coenzyme B_{12} derived from 5′(*R*)- and 5′(*S*)-(5′-$^2H_1$)ATP are due to the fact that the chirally labeled ATP is only ca. 80% optically pure at C-5′. See ref 13.
(13) Parry, R. J.; Minta, A. *J. Am. Chem. Soc.* **1982**, *104*, 871.

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the *Clostridium* enzyme catalyzes a direct displacement of triphosphate by vitamin B_{12s}. Peterkofsky[7] has reported that the *Clostridium* enzyme will catalyze the exchange of inorganic [^{32}P]triphosphate with ATP in the absence of B_{12s}. This result was interpreted as evidence for initial formation of an adenosyl-enzyme intermediate. However, Mudd[14] has pointed out a number of potential flaws in Peterkofsky's interpretation. Since our results appear to be incompatible with the formation of an adenosyl-enzyme intermediate,[15] they reinforce Mudd's concerns and suggest that the exchange observed by Peterkofsky is due to processes other than the formation of an adenosyl-enzyme.

Acknowledgment. We thank Professor Frederick Rudolph for helpful advice concerning enzyme purification, the N.I.H. (GM-26166) and The Robert A. Welch Foundation (C-729) for support, and Dr. Robert Gampe of the University of Texas Medical Center, Houston, for NMR spectra.

Supplementary Material Available: Procedures for enzyme purification and for the enzymatic preparation of coenzyme B_{12} from labeled ATP's plus NMR spectra of unlabeled coenzyme B_{12} and of coenzyme B_{12} derived from (5′-$^2H_2$)ATP (5 pages). Ordering information is given on any current masthead page.

(14) Mudd, S. H. "The Adenosyltransferases" "The Enzymes VIIIA", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1973; pp 121–152.
(15) Formation of coenzyme B_{12} via the two-step mechanism would require that one step take place retention of configuration and the other with inversion. Displacement with retention of configuration is mechanistically unlikely.

Mechanistic and Synthetic Studies of the Cyclopropyl Iminium Ion Rearrangement: An Efficient Route to Enammonium Salts and Related Enamines and Dienamines

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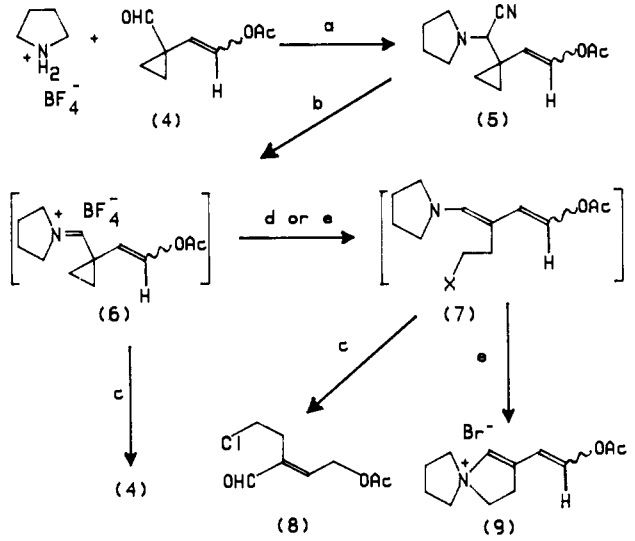
The extensive and systematic studies of Professor R. V. Stevens and co-workers established the generality and synthetic utility of the rearrangement of cyclopropyl imines under acidic catalysis at temperatures of 110 to 150 °C to provide Δ^2-pyrrolines.[1] Some evidence was accumulated which suggested that the rearrangement proceeded by a stepwise mechanism, however, no intermediates were detected to substantiate this reasonable and generally accepted hypothesis.[2,3] A number of syntheses of members of the mesembrine, amaryllidaceae, and pyrrolizidine alkaloid families have been completed by several groups utilizing this protocol.[1,3]

However, the method suffers from several limitations, the major one being the requirement for relatively vigorous acidic reaction conditions. In many instances, the success of the process depends upon the ability to remove the reactive endocyclic enamine from the reaction mixture as it is formed. Thus, there are inherent factors that reduce significantly the scope of the process, especially for applications involving relatively complex and sensitive multifunctional molecules. Therefore, we chose to investigate a process that might circumvent these difficulties and permit access to highly functionalized dienamines which were of interest as intermediates

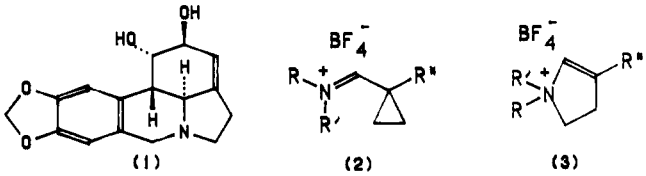
(1) Stevens, R. V. *Acc. Chem. Res.* **1977**, *10*, 193.
(2) Stevens, R. V. In "The Total Synthesis of Natural Products"; ApSimon, J., Ed.; Wiley: New York, 1977; Vol. III, p 439.
(3) A related transformation was recently reported by Wasserman and co-workers and its mechanism (also stepwise) defined: (a) Wasserman, H. H.; Dion, R. P. *Tetrahedron Lett.* **1982**, 1413. (b) Wasserman, H. H.; Dion, R. P. *Tetrahedron Lett.* **1983**, 3409.

Scheme I[a]



[a] Reagents: (a) KCN, $MgSO_4$/THF/40 °C/36 h; (b) $AgBF_4$ (1.5 equiv)/DME/room temperature; (c) H_2O; (d) LiCl (1.5 equiv)/DME/room temperature (1.5 h) then 82 °C/1 h; (e) LiBr (1.5 equiv)/CH_3CN/room temperature (4 h).

toward the amaryllidaceae alkaloid lycorine (**1**). We hypothesized that the more highly stabilized iminium ion **2**, which could conceivably be generated under mild, nonacidic conditions, would undergo more facile rearrangement to the stable cyclic enammonium salt **3**. The iminium ion rearrangement appeared to offer



the further advantages of (1) applicability to both 1° and 2° amines, (2) protection of the labile endocyclic enamine or dienamine as the readily isolable enammonium salt **3**, from which the related enamine could be regenerated upon choice of an appropriate alkyl group removable from nitrogen, and (3) substantial flexibility in the nature of the precursors to the iminium salts **2** as well as the conditions under which the intermediates **2** would be generated and the subsequent rearrangement conducted. This hypothesis proved to be correct as described below.

First, with regard to mechanism, we have been able to substantiate the stepwise nature of the transformation. Condensation of aldehyde **4** (cis/trans mixture)[4] with pyrrolidinium fluoroborate (KCN/$MgSO_4$/THF/40 °C/36 h) provided the cyanoamine **5**.[5] Treatment of **5** with $AgBF_4$ in DME (room temperature/0.75 h), as expected,[6] afforded the derived iminium ion **6** which upon addition of water regenerated **4** (Scheme I). Treatment of the solution of **6** in DME with LiCl at room temperature (1.5 h) followed by heating at reflux (1 h) resulted in generation of the intermediate enamine **7**, whose structure was established by hydrolysis to aldehyde **8**.[7] *When LiBr is utilized and the solvent*

(4) Compound **4** was prepared by the following six-step sequence. Alkylation of cyclopropyl cyanide with 3,3-dimethylallyl bromide (LDA) followed by reduction (DIBAL) and treatment of the resulting aldehyde with trimethyl orthoformate yielded the corresponding acetal. Subsequent ozonolysis, formation of the enol acetate (Et_3N/Ac_2O/DMAP), and acidic hydrolysis affords compound **4**.
(5) Direct condensation of the perchlorate salt and aldehyde by the method of Leonard and Paukstelis (Leonard, N. J.; Paukstelis, J. V. *J. Org. Chem.* **1963**, *28*, 3021) is unsuccessful.
(6) Reiber, H. G.; Stewart, T. D. *J. Am. Chem. Soc.* **1940**, *62*, 3026. Grierson, D. S.; Harris, M.; Husson, H. P. *J. Am. Chem. Soc.* **1980**, *102*, 1064.

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