benzene. Although cyclopropylidene is predicted to have a singlet ground state,[3] the cis and trans tricyclic pairs **17**,**18** and **19**,**20** which are observed here provide argument for a stepwise triplet process, indicating that spin inversion must be slow relative to hydrogen abstraction.

We are continuing to study the mechanism of this reaction, as well as its potential for the synthesis of other novel polycyclic hydrocarbons.

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Evidence for the Hydride Abstraction Mechanism in the C–H Insertion Reaction as Illustrated in the Reaction of Secondary Alkoxide with Alkylidenemethylene Carbenoid

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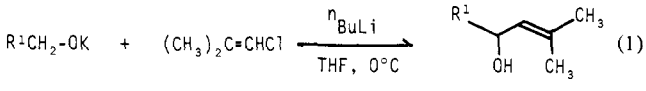
We report here the evidence for hydride abstraction–recombination mechanism in the carbenic C–H insertion reaction of alkoxides obtained by the stereochemical investigation[1] of alkylidenemethylene carbenoid ($R^1R^2C{=}C{:}\cdots MX$)[2] insertion into α-C–H bond of secondary alkoxides.

In an examination of the oxy anionic effect on the selective C–H insertion,[3] alkylidenemethylene carbenoid was inserted into the α-C–H bond of alkoxides regioselectively, as illustrated by the exclusive formation of insertion product **3** in the reaction of potassium primary alkoxide (**1**) with 1-chloro-2-methylpropene (**2**) (2.0 equiv) by the action of *n*-BuLi (2.0 equiv) in THF at 0 °C for 10 min (eq 1).



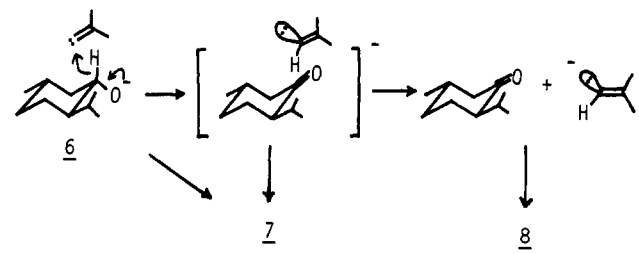
(1)

1a-d 2 3a-d

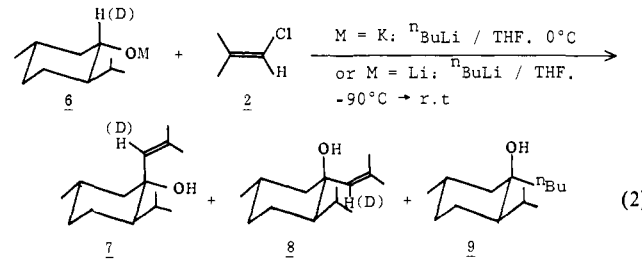
a, $R^1 = C_6H_5CH_2$, Y; 67% (42% conversion); b, $R^1 = C_6H_5$, Y; 61% (62% conversion); c, $R^1 = n\text{-}C_7H_{15}$, Y; 50% (50% conversion); d, $R^1 = 2,4,6\text{-}Me_3C_6H_2$, Y; 64% (34% conversion)

On the other hand, secondary alkoxides behaved differently and rendered important evidence for the insertion mechanism. Thus, when potassium cyclohexyl oxide was treated with **2** under similar conditions, 1-butylcyclohexanol (**5**, 17%) was obtained together with the insertion product **4** (41% yield, 43% conversion). The formation of **5** strongly suggests the intermediacy of cyclohexanone, which is most likely produced from the alkoxide by the hydride abstraction by isopropylidenemethylene carbenoid.[4] The formation of **4** is also explainable in terms of the same hydride abstraction mechanism in which the insertion may proceed nonstereospecifically. To examine this, we chose menthyl oxide (**6**) as a suitable substrate, since we found in separate experiments that menthone undergoes an exclusive equatorial attack by (2-methylpropenyl)lithium or *n*-BuLi.[5]

Scheme I



The reaction of potassium menthyl oxide (**6**, M = K) proceeded distinctively without stereospecificity to give a mixture of axial insertion product **7**, equatorial insertion product **8**, and butyl adduct **9** in the ratio of 24:4:72 (total yield 56%, 37% conversion) (eq 2). When a THF solution of lithium menthyl oxide (2.0 equiv) and **2** was treated with *n*-BuLi at −90 °C and then warmed up to a room temperature, the relative yield of **8** increased in comparison to those of **7** and **9** (7:8:9 = 47:12:41, total yield 30%). Moreover, the reaction of lithium menthyl-*1*-d_1 oxide (**6**-*d* *d* content >95%) gave **8**-*d* which contained 84% deuterium at the vinylic position.[6] Thus, it is evident that **8** was produced via the hydride abstraction–recombination mechanism. Also highly probable is that in-cage recombination between intermediates menthone and 2-methylpropenyl anion is responsible, at least partly, for the formation of axial insertion product **7** (Scheme I).



6 2 7 8 9 (2)

The reaction conditions employed here for the generation of the carbenic species suggest that the reactions proceed not through a free carbene but through a carbenoid. This can be further verified by the comparison of above reaction with that of a free (or an unencumbered) carbene[2b] generated from 2-methylpropenyl triflate (**10**); the reaction of **6** with the carbene generated from **10** and *t*-BuOK in THF gave 4-(menthyloxy)butyl 2-methylpropenyl ether (**11**) as a major product (17%),[7,8] whereas **11** was absent in the reaction of **2** with *n*-BuLi.

Primary alkoxides can also be inserted either through the concerted mechanism (path a) or through the hydride abstraction followed by a rapid recombination (path b) because no H–D scrambling was observed in the reaction of **2** with a mixture of benzyl-α,α-d_2 oxide and *p*-chlorobenzyl oxide by the action of *n*-BuLi and because butyl adduct was not formed in the reaction of primary alkoxides. However, we excluded path b for the following reason: In contrast to other primary alkoxides, the reaction of **2** with sterically hindered potassium 2,4,6-trimethylbenzyl oxide (**1d**) gave the butyl adduct (17% yield) together with insertion product **3d**. If path b were the case, the above anomaly can only be understood in terms of the steric effect of trimethylphenyl group which retards the recombination. But a competition reaction of (2-methylpropenyl)lithium with 2,4,6-

(1) (a) Kirmse, W. "Carbene Chemistry", 2nd ed.; Academic Press: New York, 1971; Chapter, 7. (b) Gasper, P. P.; Hammond, G. S. "Carbenes"; Moss, R. A., Jones, M. Jr., Eds.; Wiley: New York, 1975; Vol. II, Chapter 6.

(2) For reviews, see: (a) Stang, P. J. *Acc. Chem. Res.* **1982**, *15*, 348. (b) Stang, P. J. *Chem. Rev.* **1978**, *78*, 383. (c) Hartzler, H. D. "Carbenes"; Moss, R. A., Jones, M., Jr., Eds.; Wiley: New York, 1975; Vol. II, Chapter 2.

(3) (a) Harada, T.; Oku, A. *J. Am. Chem. Soc.* **1981**, *103*, 5965. (b) Harada, T.; Akiba, E.; Oku, A. *Ibid.* **1983**, *105*, 2771. (c) Harada, T.; Nozaki, Y.; Oku, A. *Tetrahedron Lett.* **1983**, *24*, 5665.

(4) The possibility of the fragmentation of C–H insertion product into a ketone and a vinylic anion was excluded by the following experiment: Potassium alkoxide **7** was treated with *n*-BuLi in THF at 0 °C for 30 min. Formation of neither stereoisomer **8** nor butyl adduct **9** was detected by the VPC analysis (for the structure of **7**, **8**, and **9**, refer to eq 2).

(5) Ashby, E. C.; Laemmele, J. T. *Chem. Rev.* **1975**, *75*, 521.

(6) **7**-*d* was obtained with more than 95% deuterium incorporation. A slight decrease in the deuterium content in **8**-*d* might suggest the generation of (2-methylpropenyl)lithium by the reaction of **2** with *n*-BuLi.

(7) **10** reacts with THF in the presence of alkoxides to give a ring-cleaved product. (a) Gilbert, J. C.; Weerasooriya, U. *Tetrahedron Lett.* **1980**, *21*, 2041; (b) *J. Org. Chem.* **1982**, *47*, 1837.

(8) Besides **11**, menthyl 2-methylpropenyl ether (27%) was formed together with a small amount of **7** (3%) and menthone (2%).

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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.

Acknowledgment. This work was supported partially by Grant-in-Aid for Special Project Research from the Japan Ministry of Education, Science and Culture (No. 57218013).

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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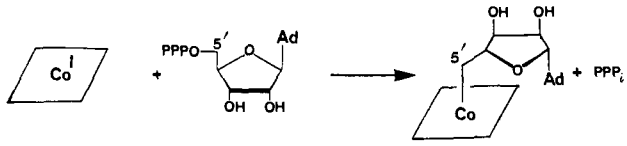
The coenzyme form of vitamin B_{12} is synthesized in living systems by the reaction of ATP with reduced vitamin B_{12} (B_{12s}) under the influence of the enzyme cob(I)alamin adenosyltransferase. This enzyme is present in homogenates of liver and kidney[1] and in extracts of Hela cells grown in tissue 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.

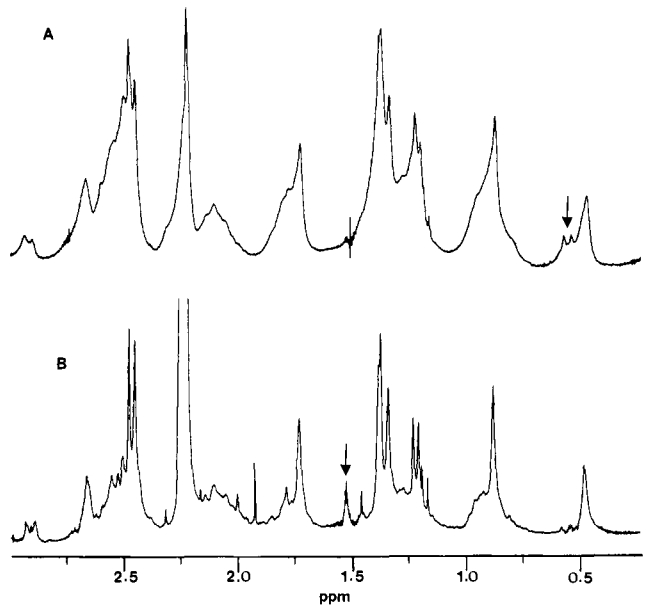


Figure 1. (A) Adenosylcobalamin derived from 5′(*R*)-(5′-$^2H_1$)ATP. (B) Adenosylcobalamin derived from 5′(*S*)-(5′-$^2H_1$)ATP. The NMR spectra were taken at 270 MHz in D_2O.

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.; Rêtey, 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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