Notes

Sterically Hindered Cyclopropanemethanol Derivative As the Mechanistic Probe in Alcohol Dehydrogenase

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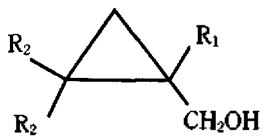
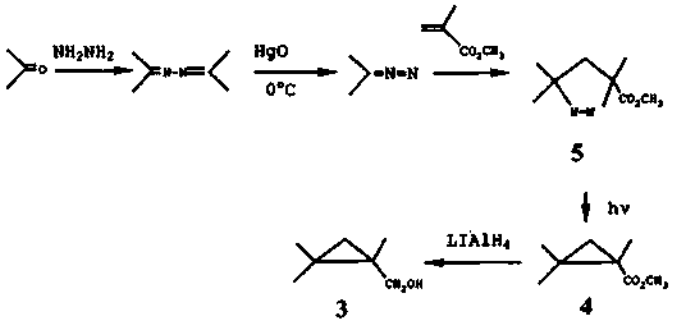
Received December 9, 1991

The Mechanism of hydrogen transfer of alcohol dehydrogenase has been controversial since Westheimer's model study of reduction reaction of 1,4-dihydropyridines for many years.[1] Most of results on the model system indicated a substantial negative charge on the hydrogen atom in the transition state.[2] Despite evidences against radical character on the mechanism of hydrogen transfer in the alcohol dehydrogenase reaction, there have been persistent reports that alcohol dehydrogenase undergoes radical-like mechanism.[3,4] For certain enzymatic or organic reaction system, cyclopropylmethyl dervatives have been used successfully to distinguish redical mechnism from hydride mechanixm because the cyclopropane ring could be rapidly opened if the radical center was adjacent to the cyclopropane ring. For example, Sucking *et al.*, analyzed the reactionn product of exo-bicyclic[4.1.0] heptan-7-ylmethanol and bicyclo[4.1.0]heptan-2-ol with horse liver alcohol dehydrogenase and they found no evidence for radical intermediates in redox reactions catalyzed by horse liver alcohol dehydrogenase. However, this approach is valid only in the case where the rate of enzyme-catalyzed hydrogen transfer is slow enough for the generated radical species to be rearranged. In addition, the enzyme could be inactivated by the attack of an nucleophilic residue of the enzyme on the cyclopropane ring with producing a ring-opened product although the ring-opening process did not involve any generation of radical species. Apart from the bicyclic system, monocyclic system such as (1) was also used as the mechanistic probe in the reaction catalyzed by alcohol dehydrogenase and the result was interpreted as the negaive evidence for a radical mechanism.



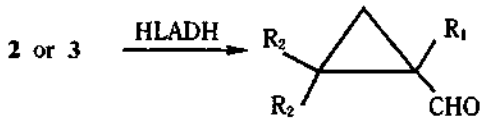
(1) $R_1=H$, $R_2=H$
(2) $R_1=CH_3$, $R_2=H$
(2) $R_1=CH_3$, $R_2=CH_3$

Since the ring-opening rate of cyclopropylmethyl radical might be radically different in the active-site of the enzyme and actually much slower than the enzymatic turnover rate of hydrogen transfer, especially because the adjacent oxygen atom of the cyclopropylmethanol may increase the stability of the nascent radical species, compound (2) and (3) were designed, which were expected to increase the ring-opening rate of the cyclopropylmethyl radical species and decrease the attack of enzymic nucleophile on the ring by imposing



Scheme 1

steric hindrance on the cyclopropane ring. Methyl substitution on the ring would put more ring strain and make the cyclopropylmethyl radical more favorable for ring-opening. In addition, we also wanted to investigate how such an imposement of steric hindrance would affect the inactivation of the enzyme. Compound (2) was readily available from Aldrich Co. and (3) was prepared by the photolysis of 3-carbomethoxy-3,5,-5-trimethyl-1-pyrazoline (5) and subsequent reduction of 1-carbomethoxy-1,2,2-trimethylcyclopropane (4) (Scheme 1).[5,6] Each alcohol, (2) or (3), was incubated with horse liver alcohol dehydrogenase in excess of acetaldehyde under the NAD cofactor regeneration system developed by Jones.[7] The control contained everything except NAD. After 24 hrs' incubation, no alcohol was remained based on alcohol was remained based on TLC. The products of reaction were isolated by several extractions with methylene chloride. The extracted product from the incubation of (2) was analyzed by GC/MS. GC result clearly indicated that compound (2) was converted to a single product of which structure was determined to be (I) by comparison of its mass spectrum with the authentic sample prepared independently.[8]



(I) $R_1=CH_3$, $R_2=H$
(II) $R_1=R_2=CH_3$

The same analytical procedure was tried for the incubation product of (3). However, compound (3) and its oxidation product was thermally unstable and decomposed under the employed GC analytical condition. Since it was clear that (3) was converted into a single product during the incubation based on TLC, the incubation product was purified by silicagel column chromatography (hexane : ethylacetate = 10 : 1). Examination of the NMR spectrum of the purified product revealed that the cyclopropane ring was still intact.[9] Therefore, horse liver alcohol dehydrogenase did not open th cyclopropane ring during the enzymatic turnover when (2) or (3) was used as a substrate. Consistent with previous reports, we did not find any evidence for radical intermediates in redox reactions catalyzed by horse liver alcohol dehydro-

genase. It is strongly beliveved that the mechanism of alcohol dehydrogenase reaction is hydride-like rather than radical mechanism.

Since cyclopropylmethanol derivatives such as (1) were known as latent inhibitors of horse liver alcohol dehydrogenase,[10] it seemed to be interesting to know whether or not such highly strained compounds as (2) and (3) inhibited the enzyme. Compound (2) or (3) was incubated with horse liver alcohol dehydrogenase and the activity was checked following the exactly same procedure as reported previously.[10] Surprisingly, the enzyme was not inhibited by (2) or (3) at all although their K_m and k_{cat} values determined by Lineweaver-Burk plot were similar to (1).[11] Therefore, the steric hindrance exerted by methyl substitutions on the cyclopropoane ring effectively prevents the enzymic residue from attacking the ring in S_N2 fashion, Compound (2) and (3) are expected to be useful as the mechanistic probe avoiding complications resulting from the nucleophilic ring-opening in alcohol dehydrogenase reactions as well as other enzymatic reactions.

Acknowledgement. This research was supported by Academic Foundation from Chonnam National University and the Korea Science and Engineering Foundation. Author is indebted to Miss S. Kim for running kinetic experiments.

References

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6. A stirred solution of 2.0 g of (5) in 500 m*l* of pentane was irradiated through quartz for 4 hr with Hanovia lamp. After the starting material disappeared based on NMR, the pentane was evaporated to produce 1.4 g of (4) (83%). Compound (4) (1.0 g) was dissolved in 30 m*l* ether and 10 equivalents of lithium aluminium hydride was added. The reaction mixture ws stirred for 30 min and standard workup porduced 0.7 g of (3) (90%).
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8. The authentic sample of compound (I) was prepared by oxidation of (2) by pyridiniumchlorochromate.
9. NMR specturm of compound (II) (250 MHz, $CDCl_3$) : 9.21 (s, 1H, -CHO), 1.48 (d, 5.0 Hz, 1H, cyclopropyl proton), 1.30 (s, 3H, $-CH_3$), 1.20 (s, 3H, $-CH_3$), 1.08 (s, 3H, $-CH_3$), 0.82 (d, 5.0 Hz, 1H, cyclopropyl proton).
10. I. MacInnes, D. C. Nonhebel, S. T. Orszulik, and C. J. Suckling, *J. Chem. Soc. Perk. Trans. I.*, 2777 (1983).
11. K_m values and k_{cat} values of (1), (2), (3) were 6.89×10^{-5} M, 70.4×10^{-5} M, 140×10^{-5} M, 4.24 S^{-1}, 7.79 S^{-1}, 10.03 S^{-1} respectively.

Selective Reduction by Lithium Bis- or Tris(dialkylamino)aluminum Hydrides. VI. One-Pot Conversion of Primary Carboxamides into Aldehydes *via* Stepwise Treatment with Diisobutylaluminum Hydride and Lithium Tris(diethylamino)aluminum Hydride[1]

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Received December 19, 1991

In the previous communications[2], we reported a new methodology for direct transformation of both aliphatic and aromatic primary carboxamides to the corresponding aldehydes by utilizing lithium tris(diethylamino)aluminum hydride (LTDEA) and lithium tripiperidinoaluminum hydride (LTPDA). These results led us to think over the reaction pathway. Consequently, we found an alternative simple method for such transformation. Herein, we now report this reduction of primary carboxamides to the corresponding aldehydes by using diisobutylaluminum hydride (DIBAH) and LTDEA.

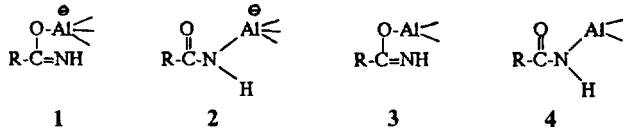
One of the most important features to be considered in this reaction seems to be the reaction intermediates which are formed after the hydrogen evolution from the reaction of primary amide and reagent (Scheme 1). In cases where LTDEA is utilized, the intermediate would be **1** or **2** and where DIBAH is used, the intermediate would be **3** or **4**.

When compared **1** with **3** (or, **2** with **4**), we could expect that the intermediate without negative charge on aluminum would be more favorable to reduction through the more efficient electron-withdrawing effect.

Therfore, we added 1 equiv of DIBAH to the solution of carboxamides to form the intermediate **3** (or **4**) at 0℃[3], and treated it with excess LTDEA at room temperature consecutively in the hope of resulting in the rate and yield enhancement (Eq 1).

$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}NH_2 + HAl(i\text{-}Bu)_2 \xrightarrow[0℃]{THF} \mathbf{3}\ (\text{or}\ \mathbf{4}) \xrightarrow[rt]{\text{excess LTDEA}} \xrightarrow{H_3O^+} RCHO$$

The system reduces aliphatic primary carboxamides readily at room temperature to provide the corresponding aldehydes in yields of 50-70%, as shown in Table 1. The yields of aldehydes appear to be varying with the structure of



1 **2** **3** **4**

Scheme 1