Stereochemistry of the Coenzyme B_{12}-mediated Rearrangement of 2-Aminoethan-1-ol by Ethanolamine Ammonia-lyase

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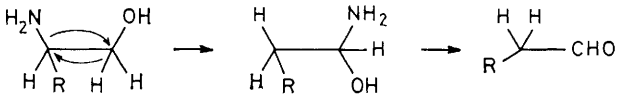
Reaction kinetics and ^2H n.m.r. spectra of reaction products show independently that the coenzyme B_{12}-mediated rearrangement of 2-aminoethan-1-ol by the enzyme ethanolamine ammonia-lyase proceeds with migration of the (1*S*)-hydrogen.

Rearrangements involving coenzyme B_{12} are of considerable current interest.[1] The enzyme ethanolamine ammonia-lyase catalyses one such reaction, the natural substrate 2-aminoethan-1-ol (**1**, R=H) rearranging in the process to acetaldehyde (**3**, R=H) as shown in Scheme 1. This enzyme also catalyses the rearrangement of (2*S*)- and (2*R*)-2-aminopropan-1-ols (**1**, R=Me) to propionaldehyde (**3**, R=Me) and the stereochemistry of the rearrangement has been examined for these substrates.[2] The stereochemistry of the rearrangement of the natural substrate, 2-aminoethan-1-ol, is of particular interest in view of a report[3] that the centre C-2 becomes racemic in the process, an unusual result for an enzyme-catalysed reaction. Since we have recently synthesised[4] samples of 2-aminoethan-1-ol stereospecifically labelled with deuterium at C-1, we determined to study the stereochemistry of the rearrangement at the migrating centre C-1.



(1) (2) (3)

Scheme 1

2-Aminoethan-1-ol (**4**, R=H), [1,1-$^2H_2$]-2-aminoethan-1-ol (**4**, R=H, $H_S=H_R=^2H$),† (1*R*)-[1-$^2H_1$]-2-aminoethan-1-ol (**4**, R=H, $H_R=^2H$),[4] and (1*S*, 2*RS*)-[1,2-$^2H_2$]-2-aminoethan-1-ol (**4**, R=$H_S=^2H$)[4] were separately incubated at 24 °C with ethanolamine ammonia-lyase (EC.4.3.1.7)[5] and coenzyme B_{12} to produce acetaldehyde. This was reduced *in situ* by an excess of yeast alcohol dehydrogenase and NADH so that the rates of the rearrangements could be measured by following the decrease in the u.v. absorbance due to NADH at 340 nm. Taking the rate for 2-aminoethan-1-ol (**4**, R=H) as $k_H=1$, the values of k_H/k_D for the other substrates were, (1*R*)-[1-$^2H_1$]-2-aminoethan-1-ol (**4**, R=H, $H_R=^2H$) 0.98; [1,1-$^2H_2$]-2-aminoethan-1-ol (**4**, R=H, $H_S=H_R=^2H$) 7.7; and (1*S*, 2*RS*)-[1,2-$^2H_2$]-2-aminoethan-1-ol (**4**, R=$H_S=^2H$) 8.3. Since it is the compounds with deuterium replacing H_S which show an isotope effect, it is evident that this is the hydrogen which migrates in the rearrangement.

These results were confirmed independently by conducting the above reactions on a larger scale and isolating the ethanol produced as its 3,5-dinitrobenzoate (**5**). The ^2H n.m.r.

† Prepared by reduction of ethyl glycinate hydrochloride with $LiAl^2H_4$ and shown to have ^2H only at C-1 by ^2H n.m.r. spectroscopy of the *N*,*O*-dicamphanoyl derivative.

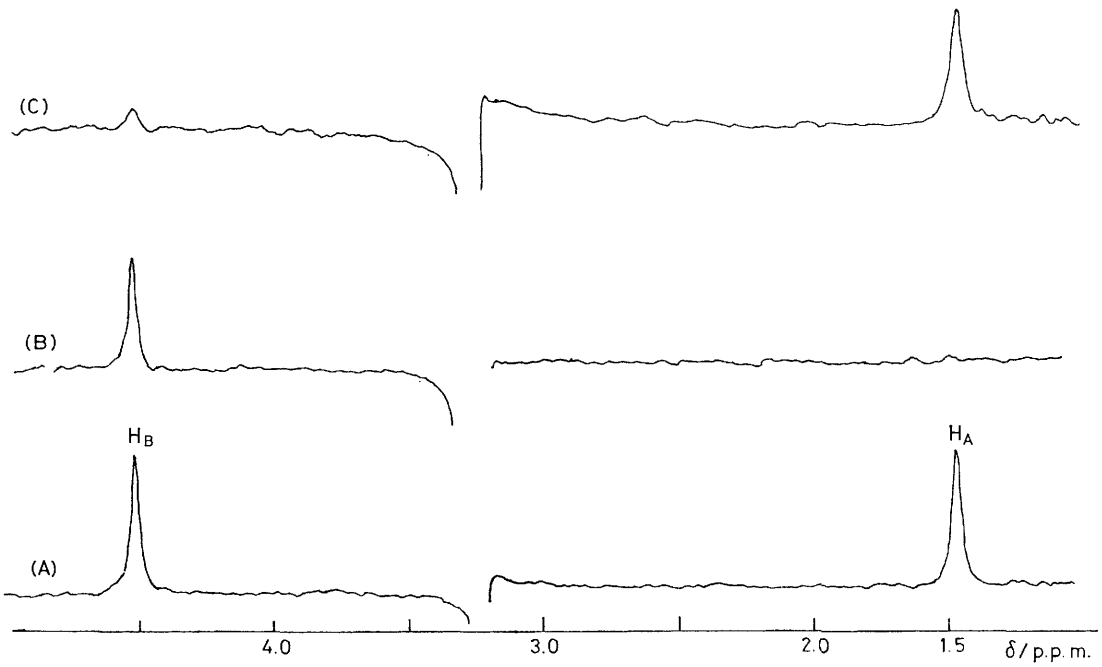
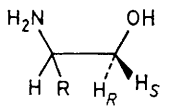
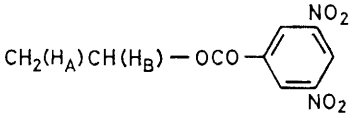


Figure 1. ^2H N.m.r. spectra (55 MHz) in C^2HCl_3 of the product (**5**) derived ultimately from the ethanolamine ammonia-lyase catalysed rearrangement of (A) [1,1-$^2H_2$]-2-aminoethan-1-ol (**4**, R=H, $H_S=H_R=^2H$), (B) (1*R*)-[1-$^2H_1$]-2-aminoethan-1-ol (**4**, R=H, $H_R=^2H$), and (C) (1*S*, 2*RS*)-[1,2-$^2H_2$]-2-aminoethan-1-ol (**4**, R=$H_S=^2H$).



(4)



(5)

spectra‡ of the 3,5-dinitrobenzoates (**5**) are shown in Figure 1. It is evident from this that, whereas the (1*R*)-deuterium remains on the carbon which becomes the CH_2–O group of the product, the (1*S*)-deuterium has migrated to the carbon which becomes the methyl group in the product.§

These results indicate complete stereospecificity in the rearrangement at the carbon atom from which the hydrogen migrates. It is the (1*S*)-hydrogen which migrates in the process and any mechanism for the rearrangement must reconcile this fact with the apparent racemisation at the carbon atom receiving the migrating hydrogen.

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‡ Residual ethanol in commercial NADH made ^2H n.m.r. spectroscopy more reliable than ^1H n.m.r. spectroscopy as an assay for the rearrangement.

§ The small deuterium signal in the $-CH_2O-$ region in Figure 1 (C) might best be explained by a small amount of racemisation in the synthesis of the (1*S*)-isomer. The isotope effect in the rearrangement would exacerbate the effect of this to give a detectable signal in the product. Any racemisation in the parallel synthesis of the (1*R*)-isomer would be obscured by the same isotope effect and so might account for the very clean spectrum in Figure 1 (B).

References

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