THE STEREOCHEMISTRY OF THE CONVERSION OF PROPANEDIOL TO PROPIONALDEHYDE[1]

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Dioldehydrase, an enzyme which requires a cobamide coenzyme, catalyzes the conversion of 1,2-propanediol to propionaldehyde and ethylene glycol to acetaldehyde (Abeles, R.H. and Lee, H.A., 1961). These reactions involve a 1,2-hydrogen shift in which no exchange occurs with the hydrogen of the solvent (Brownstein, A.M. and Abeles, R.H., 1961). Experiments were carried out to establish whether one of the two hydrogens of C-1 of propanediol is preferentially transferred. For this purpose we synthesized stereospecifically labeled 1,2-propanediol-1-d_1 by the reduction of D- and L-lactaldehyde with DPND and ADH[3]. We expected that the conversion of these diols would lead to the formation of either α-deutero-propionaldehyde or to C-1-deutero-propionaldehyde. Instead, we found that D-propanediol-1-d_1 gave rise to α-deutero-propionaldehyde and L-propanediol to C-1-deutero-propionaldehyde.

The following equations illustrate the reactions which were carried out:

$$\underset{\text{D-lactaldehyde}}{CH_3\text{-}CHOH\text{-}CHO} + CH_3CD_2OH \xrightarrow[DPN]{ADH} \underset{\text{D-1,2-propanediol-1-}d_1}{CH_3\text{-}CHOH\text{-}CHDOH} + CH_3CHO$$

$$\underset{\text{D-1,2-propanediol-1-}d_1}{CH_3\text{-}CHOH\text{-}CHDOH} \xrightarrow[DBCC]{\text{Diol dehydrase}} \underset{\text{propionaldehyde-α-d}}{CH_3\text{-}CHD\text{-}CHO}$$

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[3]The following abbreviations are used: DPND, α-deutero-dihydro-pyridine mononucleotide; ADH, liver alcohol dehydrogenase; DBCC, dimethyl benzimidazoyl-cobamide coenzymes.

When the same series of reactions was carried out with L-lactaldehyde, CH_3-CH_2-CDO was obtained.

The deuterium location in propionaldehyde was established from the NMR spectrum of propionaldehyde-2-4-dinitrophenylhydrazone. These spectra are shown in Fig. 1. Spectrum B is the spectrum of propionaldehyde-2,4-dinitro-

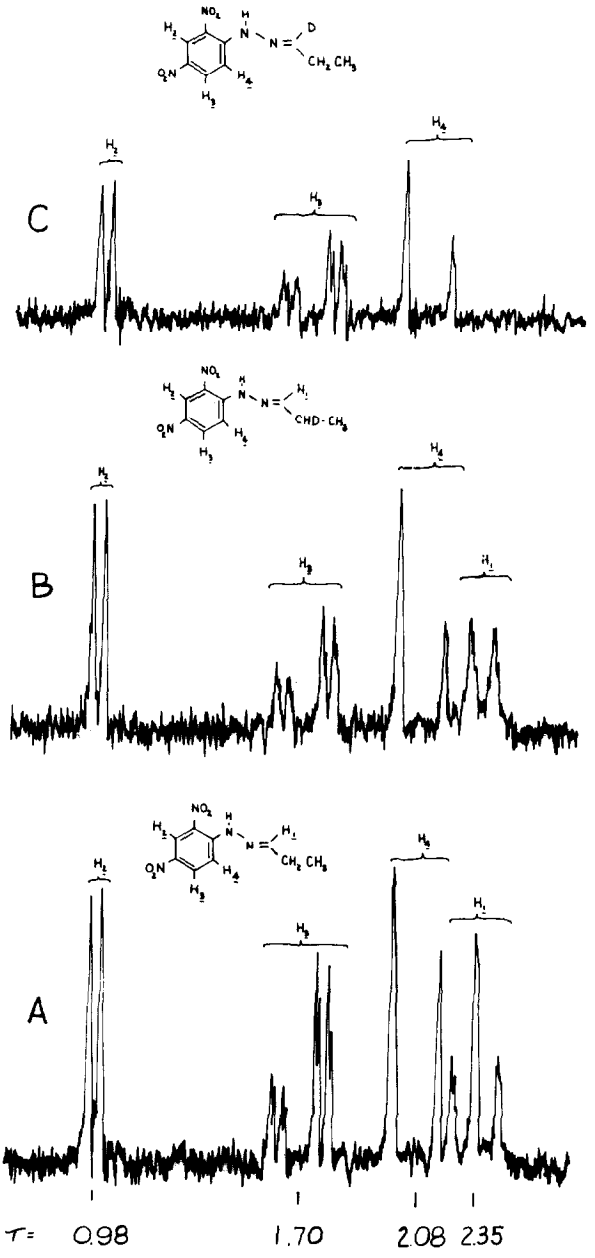


Fig. 1 NMR spectrum of propionaldehyde-2,4-DNP. Propionaldehyde-2, 4-DNP (A), monodeutero-propionaldehyde-P. 2,4-DNP from L-lactaldehyde (B), from D-lactaldehyde (C). Spectra taken in methylene bromide.

phenylhydrazone derived from D-propanediol and spectrum C that derived from L-propanediol. The triplet at $\tau = 2.35$ in A becomes a broadened doublet in B and is absent in C. From this we conclude that the deuterium is at the methylene group in B and at C-1 in C.

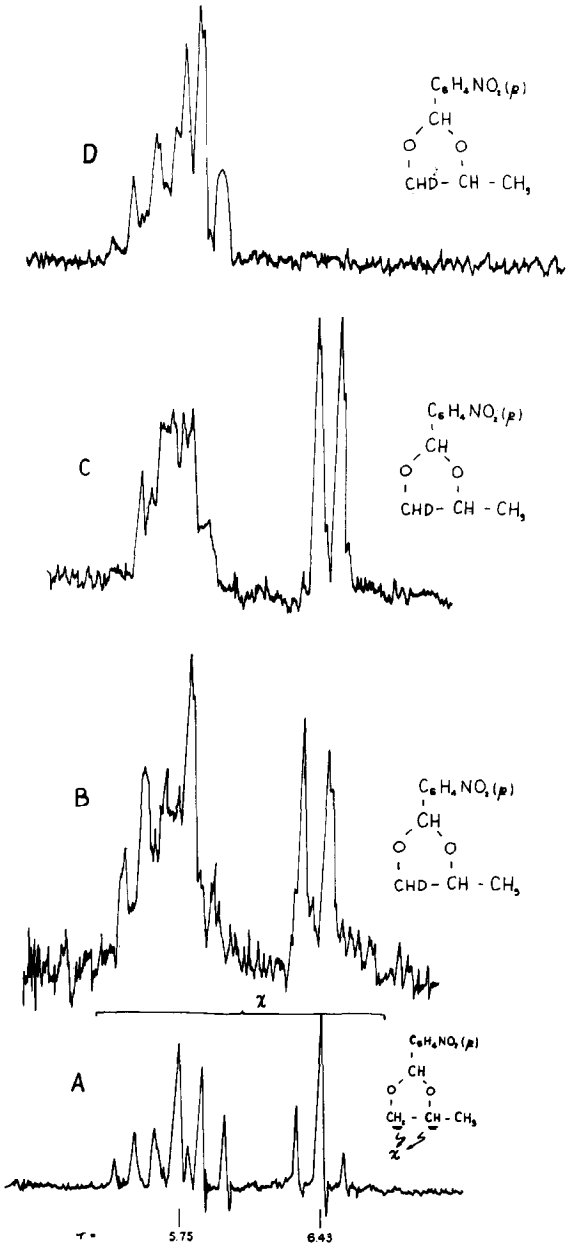


Fig. 2 NMR spectrum of p-nitrobenzaldehyde acetals of 1,2 propanediol. (A) 1,2-propanediol, (B) 1,2-propanediol prepared by $LiAlD_4$ reduction of D,L-lactaldehyde, (C) 1,2-propanediol-1-d_1 enzymatically from D-lactaldehyde, (D) 1,2-propanediol-d_1 enzymatically from L-lactaldehyde. Spectra taken in carbon tetrachloride.

The kinetics of propionaldehyde formation support the NMR data. The conversion of D-propanediol-1-d_1 to propionaldehyde proceeds at 1/12 the rate of the non-deuterated substrate. This isotope effect shows that this reaction involves a deuterium transfer. Deutero-L-propanediol reacts as fast as the non-isotopic substrate. Therefore, the conversion of L-propanediol-1-d_1 involves the transfer of hydrogen rather than deuterium.

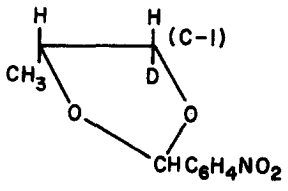
Two different explanations can be offered for the results obtained. These are:

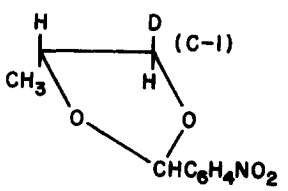
1) The stereospecificity of the reduction of lactaldehyde by ADH is controlled by the configuration of the α-carbon so that the configuration at C-1 of the resulting monodeutero D- and L-propanediol are not identical, and the two monodeuterodiols are enantiomorphs. If dioldehydrase is specific for one of the hydrogens at C-1, the results described here would be obtained.

2) D and L-propanediol produced from the corresponding lactaldehyde have the same configuration at C-1, i.e. they are diastereoisomers. The conversion of the diols to propionaldehyde proceeds so that one hydrogen is transferred when the L-isomer is the substrate and the other when the D-isomer is the substrate.

In order to decide which of the two alternatives applies we established whether the two monodeuterated diols have the same configuration at C-1. This was done by converting the diols to the p-nitrobenzaldehyde acetals and examining the NMR spectra. The acetals can have the following configurations:

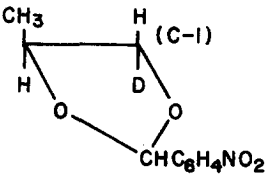
from D-lactaldehyde →

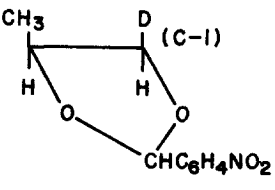




C C'

from L-lactaldehyde →





D D'

If D- and L-propanediol-1-d_1 have the same configuration at C-1, structures C and D or C' and D' would be obtained. The spectra of C vs. D and C' vs. D' will be different since the vicinal protons are trans in D and cis in C. If the configurations at C-1 are opposite, C and D' or C' and D would result. The spectra of C vs. D' and C' vs. D will be identical. The results obtained are shown in Fig. 3. Since the spectra are clearly different, it can be concluded that the reduction of D- and L-lactaldehyde by ADH leads to D- and L-propanediol with identical configurations at C-1. These results lead to the further conclusion that, in the conversion of propanediol to propionaldehyde one of the hydrogens of C-1 is transferred when L-diol is the substrate and the other when the D-isomer is the substrate.

The steric course of the conversion of propanediol to propionaldehyde can be accounted for if one assumes that interaction of enzyme and substrate involves the binding of the two hydroxyl groups at a specific site and the

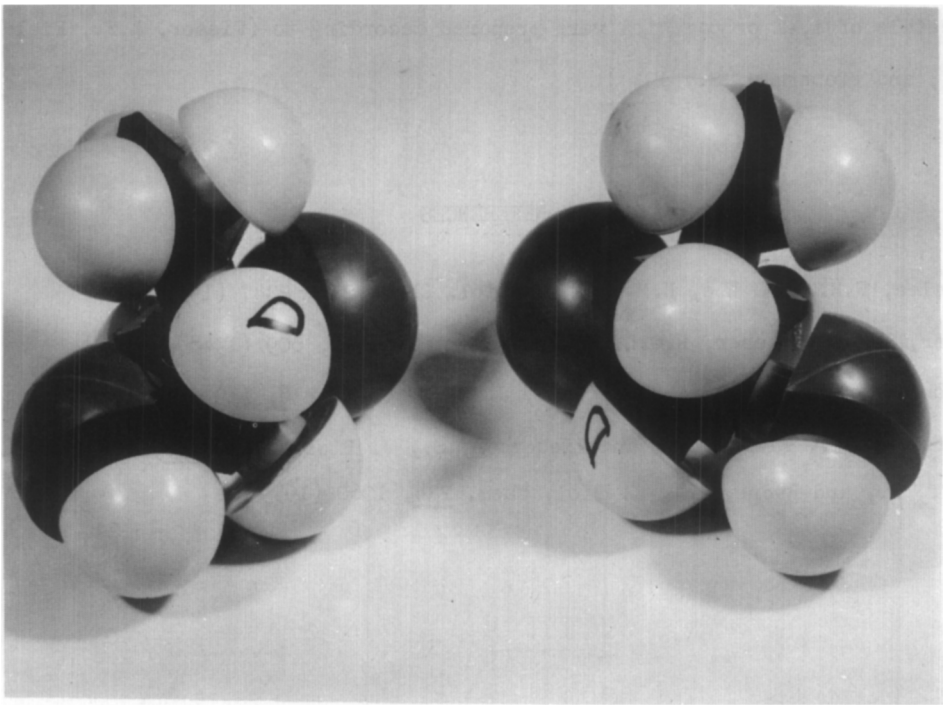


Fig. 3 Molecular models of D- and L-1,2-propanediol-1-d_1. Deuterium atoms are designated by D.

interaction of the methyl group with a specific area of the protein. Fig. 3 shows the two isomers of propanediol in one possible configuration which they would assume when bound to the enzyme in this manner. It can be seen that the position occupied by deuterium in one molecule is occupied by hydrogen in the other. Therefore, if, for any given mechanism of hydrogen transfer, a deuterium shift occurs with one isomer, a hydrogen shift occurs with the other.

Materials and Methods: Dioldehydrase was prepared and assayed as previously described (Lee, H.A. and Abeles, R. H., 1963). Ethanol-1-d_2 was obtained from New England Nuclear Corp. D- and L-lactaldehyde were prepared by ninhydrin oxidation of D- and L-threonine (Huff, E. and Rudney, H., 1959). Lactaldehyde was converted to propanediol by reduction with ethanol-1-d_2 in the presence of ADH and catalytic amounts of DPN. L-propanediol-1-d_1 had a specific rotation $[\alpha]_D^{25} = +23.3^\circ$ (3.3g/100 ml) and for D-propanediol-1-d_1 $[\alpha]_D^{25} = -21.3^\circ$ (2.3g/100 ml). Previously reported values for L-propanediol $[\alpha]_D^{25} = +20.1^\circ$ (7.5g/100 ml) (Baer, E. and Fischer, H.O.L., 1948). p-nitrobenzaldehyde acetals of 1,-2 propanediol were prepared according to (Fieser, L.F., Fields, M., and Lieberman, S., 1944).

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