exo-Bicyclo[4.1.0]heptane-7-methanol: a Novel Latent Inhibitor of Liver Alcohol Dehydrogenase

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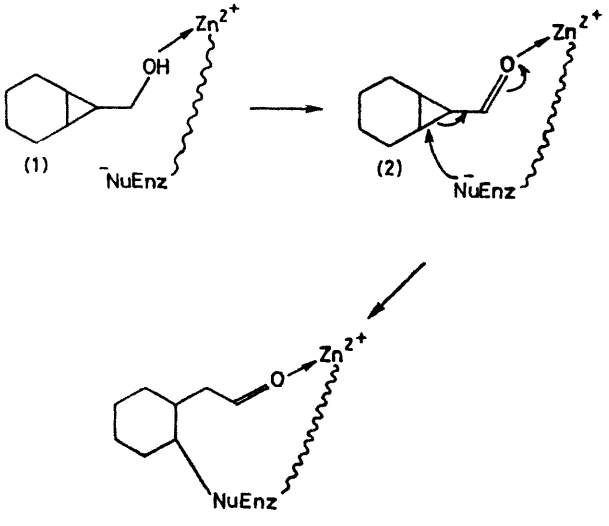
Summary Oxidation of *exo*-bicyclo[4 1 0]heptane-7-methanol by horse liver alcohol dehydrogenase and NAD^+ leads to the irreversible inactivation of the enzyme and in the presence of NADH, the enzyme catalyses the slow solvolytic ring-opening of the substrate

OUR previous studies of the inhibition of horse liver alcohol dehydrogenase (HLADH, E C 1 1 1 1) showed that 3-ethylthioprop-2-en-1-ol is a potent latent-inhibitor of the enzyme *via* ethanethiol liberated by the enzyme-catalysed hydrolysis of the oxidised substrate [1,2] In an attempt to elucidate the mechanism of hydrogen transfer in redox reactions mediated by HLADH[3] we began a study of the enzyme chemistry of bicyclic cyclopropylmethanols It was also possible that such substrates might act as enzyme inhibitors *via homo*-Michael addition to the cyclopropylaldehyde (Scheme 1) [4,5] We have found that *exo*-bicyclo-[4 1 0]heptane-7-methanol (**1**) causes inhibition of HLADH by at least two pathways

The substrate (**1**) was synthesised *via* copper-catalysed addition of ethyl diazoacetate to cyclohexene, hydrolysis of the ester formed a mixture of *exo*- and *endo*-acids which were separated by vacuum sublimation, the minor *endo*-isomer [m p 77—78 °C (lit [6] 77—78 °C)] being the more volatile Reduction of the *exo*-acid [m p 97—98 °C (lit [6] 95—96 °C)] with lithium aluminium hydride in ether afforded the alcohol (**1**) [7] The corresponding aldehyde[8] (**2**) was obtained by oxidation of (**1**) with chromium trioxide–pyridine complex in dichloromethane Before use in enzyme assays, both oxidised and reduced substrates were purified by preparative g l c on 20% Apiezon (20 ft, 160 °C)

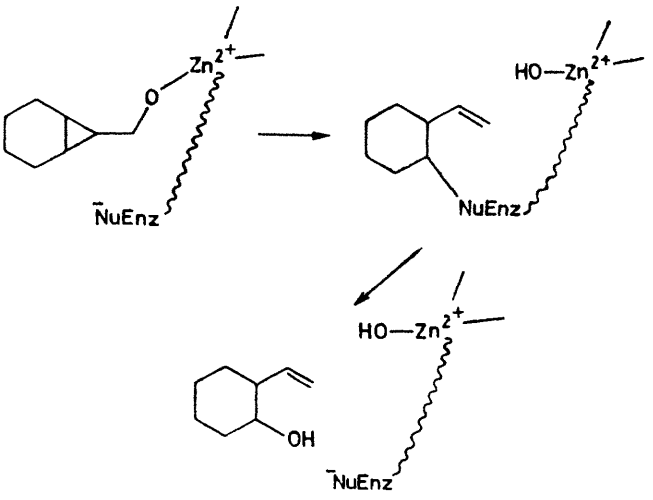


SCHEME 1

Oxidation of the alcohol (**1**) by HLADH (7.5×10^{-5}M) and NAD^+ (1.5×10^{-3}M) in 0·1M phosphate buffer (pH 9) led to rapid inhibition of the enzyme, at 30 °C, in the presence of 1.62×10^{-3}M (**1**) the half-life of the enzyme was 2 min Inhibition could not be reversed by either addition of excess of ethanol or gel filtration through Sephadex G10 The aldehyde (**2**) similarly caused inhibition of HLADH, in the presence of either oxidised or reduced coenzyme, which

likewise was irreversible. In each case, the first portion of the inhibition reaction (*ca.* 2 half-lives) was first order with respect to the enzyme and substrate, but subsequently inhibition became slower. Since inhibition is irreversible, this behaviour suggests that more than one enzyme nucleophile attacks different molecules of the substrate. Similar kinetic behaviour has been observed in the inhibition of HLADH by iodoacetate.[9]

An unusual and surprising feature of the interaction of HLADH with the alcohol (**1**) is that inhibition also occurred more slowly in the presence of the reduced coenzyme NADH. The half-life of $9{\cdot}4 \times 10^{-9}$M HLADH at pH 9 in the presence of $1{\cdot}7 \times 10^{-4}$M NADH and $1{\cdot}6 \times 10^{-3}$M (**1**) was about 8 min. Since the alcohol cannot be oxidised, the enzyme must have activated the substrate to a non-natural reaction, albeit a slow one. No reaction occurred in a control experiment from which the coenzyme was omitted. From a preparative scale reaction, the products were isolated after 3 days by continuous extraction. In addition to unchanged starting material, a small quantity (*ca.* 5—10%) of 2-vinylcyclohexanol[10] was identifiable by ^{1}H n.m.r. and g.l.c. The origin of this product can be rationalised bearing in mind that HLADH possesses a zinc dication that acts as a Lewis acid at its active site.[11] Co-ordination of the anion of (**1**) to Zn^{2+} could lead to sufficient polarisation of the C–O bond to promote nucleophilic ring-opening by the enzyme and C–O bond cleavage (Scheme 2). In this case, a small quantity of the inhibitor was hydrolysed, affording the observed product.[1,2] Cyclopropylmethanols have been shown to undergo Lewis acid-mediated ring-opening to homoallyl halides in non-enzymic reactions.[12] Since the second of the two inhibition processes is partially reversible, as shown by the production of 2-vinylcyclohexanol and by gel filtration, it seems likely that different enzyme nucleophiles are involved in each case. It will be necessary to carry out labelling experiments and protein degradation to settle this point.



SCHEME 2.

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