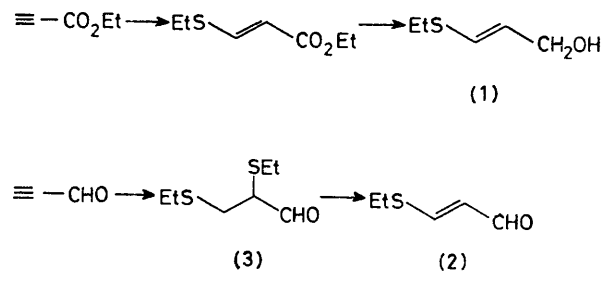
Latent Inhibition of Liver Alcohol Dehydrogenase by a Substituted Allyl Alcohol

By DAVID SCHORSTEIN and COLIN J. SUCKLING*
(*Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow* G1 1XL)
and ROGER WRIGGLESWORTH
(*Wellcome Research Laboratories, Beckenham, Kent*)

Summary 3-Ethylthioprop-2-en-1-ol is a substrate for liver alcohol dehydrogenase and also causes time-dependent inactivation of the enzyme.

'SUICIDE' inhibitors are well known for several classes of enzyme,[1-3] but not for pyridine nucleotide-dependent dehydrogenases. As part of a study[4] of latent inhibitors of liver alcohol dehydrogenase (LADH, E.C. 1.1.1.1.) we have investigated the reaction of the enzyme with substituted allyl alcohols. Allyl alcohol itself is a weak latent inhibitor of LADH,[5] presumably through addition of an enzyme nucleophile to the oxidation product, acrolein. LADH has also been shown to catalyse the reversible isomerisation of 2-*t*,6-*t*-farnesal and 2-*c*,6-*t*-farnesal.[6] A plausible mechanism again involves Michael addition to an α,β-unsaturated aldehyde to yield an adduct which can decompose to form either isomer. We reasoned that if an alternative leaving group were available in the adduct, loss of it instead of the enzyme nucleophile might cause irreversible inhibition of the enzyme. Accordingly, we prepared 3-ethylthioprop-2-en-1-ol (**1**) and the corresponding aldehyde (**2**) for testing as LADH inhibitors.

Addition of ethanethiol to ethyl propiolate (Scheme 1) afforded an α,β-unsaturated ester adduct (86%) which,



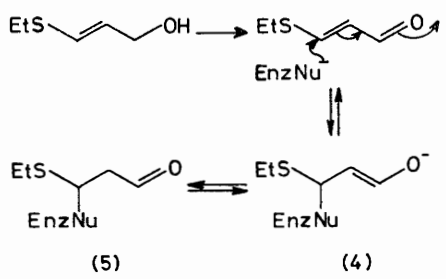
SCHEME 1

on reduction with lithium aluminium hydride, yielded (**1**) (56%). *trans*-1,1-Dideuterio-(**1**) was prepared by lithium aluminium deuteride reduction of the ester. The *trans*-aldehyde (**2**) was available by addition of ethanethiol to propiolaldehyde *via* (**3**) which was converted into (**2**) by treatment with mercuric oxide in chloroform (46% overall).

Incubation of (**1**)† (10^{-5}–10^{-3} M) with LADH ($3{\cdot}75 \times 10^{-7}$ M) and NAD^+ ($1{\cdot}5 \times 10^{-3}$ M) in phosphate buffer (pH 9·0; 0·1 M) at 25 °C caused time-dependent inactivation of the enzyme. The following results show that inhibition is due to the reaction of the enzyme with the

† Analytically pure samples of (**1**) were used in inhibition experiments.

oxidation product (**2**) or (**1**). No inactivation occurred in the absence of co-factor. Inhibition was not due to a solvolysis product of (**1**) because preincubation of (**1**) in the presence of NAD^+ followed by addition of LADH produced inactivation at the same rate as previously observed. That inhibition was taking place at the active site was suggested by the ability of substrate, ethanol, to abolish or to protect against inhibition and by the kinetic behaviour of the reaction. The rate of inactivation was proportional to the concentration of (**1**) at low concentrations but independent of it at high concentrations. In the early stages of the reaction, the rate of inactivation followed pseudo-first-order kinetics (k_{obs} $2{\cdot}9 \times 10^{-3}$ s^{-1}; [**1**] = 10^{-5} M). This rate subsequently decreased and finally a minimum activity was reached the magnitude of which depended upon the initial concentration of (**1**). A small primary kinetic isotope effect (k_H/k_D 1·4) for the rate of inhibition indicates that oxidation is a prerequisite of inactivation. If this is so, then the aldehyde (**2**) should also be an inhibitor and this was found to be the case. The aldehyde (**2**) is stable to the reaction conditions in the absence of LADH and inhibits the enzyme too rapidly at 10^{-5} M for measurement of a rate constant. Addition of saturating concentrations of ethanol to an LADH sample partially inactivated by (**2**) not only halted inhibition but also promoted the immediate slow recovery of activity as shown by an increasing rate of NADH production. We therefore conclude that (**1**) is a true latent inhibitor of LADH.



SCHEME 2

A mechanism that accommodates the above results is shown in Scheme 2. Enzyme-catalysed oxidation of (**1**) yields (**2**) which undergoes Michael addition yielding (**4**) and, after protonation, (**5**). Surprisingly, on either prolonged incubation (24 h) with (**1**) or (**2**), or on dialysis or gel filtration, the enzyme recovered activity. Reactivation of a covalently inhibited enzyme by hydrolysis has been reported[2,7] by Abeles *et al.* and the LADH reaction with (**1**) or (**2**) is similar in this respect. Unfortunately a detailed kinetic analysis of the recovery of LADH activity is difficult because ethanethiol, a likely final product, is a competitive inhibitor of LADH.[8]

The award of an S.R.C. C.A.S.E. Studentship (to D.S.) is acknowledged.

(*Received*, 16*th June* 1978; *Com.* 636.)

[1] R. R. Rando, *Science*, 1974, **185**, 320.
[2] R. H. Abeles and A. L. Maycock, *Accounts Chem. Res.*, 1976, **9**, 313.
[3] C. Walsh in 'Horizons in Biochemistry and Biophysics,' Vol. 3, ed.E. Quagliariello, Addison-Wesley, New York, 1977, p. 36.
[4] D. E. Schorstein, C. J. Suckling and R. Wrigglesworth, *J. Chem. Research* (*S*), 1978, 264.
[5] R. R. Rando, *Nature New. Biol.*, 1972, **237**, 52.
[6] C. Capillini, A. Corbella, P. Gariboldi, and G. Jommi, *Bio-org. Chem.*, 1976, **5**, 129.
[7] A. L. Maycock, R. H. Suva, and R. H. Abeles, *J. Amer. Chem. Soc.*, 1975, **97**, 5613.
[8] D. L. Morris, Ph.D. Thesis, University of Edinburgh, 1973.