Latent Inhibitors. Part 2.[1] Allylic Inhibitors of Alcohol Dehydrogenase

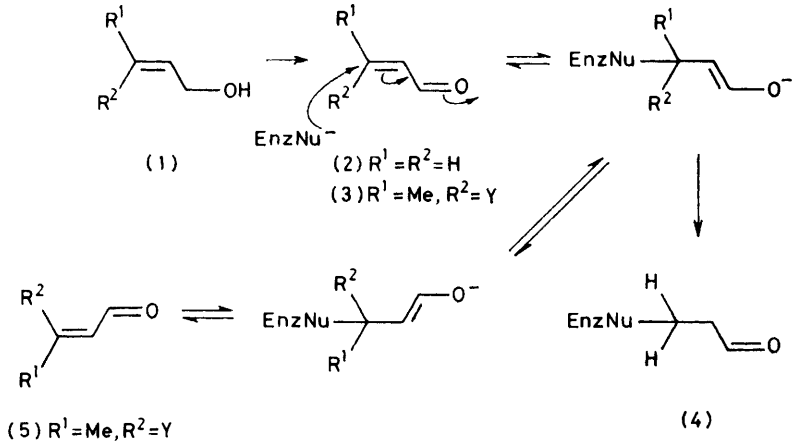
By **Iain MacInnes, David E. Schorstein,** and **Colin J. Suckling,*** Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow G1 1XL
Roger Wrigglesworth, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent

The syntheses of a number of 3-substituted prop-2-en-1-ols and -1-als, required for studying the latent inhibition of horse liver alcohol dehydrogenase (E.C. 1.1.1.1), are described. Substituents were chosen to cover a range of alkoxide, phenolate, thiolate, and halide leaving groups. Of the compounds studied, only 3-ethylthioprop-2-en-1-ol proved to be a latent inhibitor through oxidation to the corresponding aldehyde, catalysed by the enzyme. The persistence of inhibition caused by this inhibitor is shown, by product studies and kinetic measurements, to be due to ethanethiol, formed by an enzyme-catalysed hydrolysis of the aldehyde.

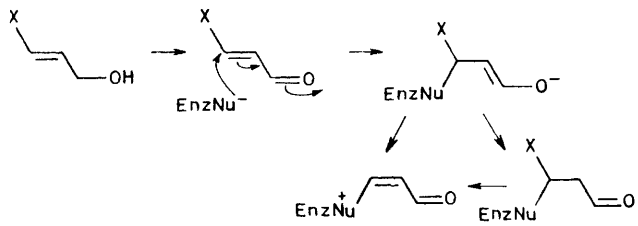
FEW latent inhibitors of nicotinamide-dependent dehydrogenases are known. However, a possible approach to the chemotherapy of some parasitic diseases is through the inhibition of dehydrogenases which are important in energy production in certain pathogens such as helminths *e.g.* ascaris. As a pilot study to discover and evaluate techniques for the latent inhibition of such dehydrogenases, we have studied horse liver alcohol dehydrogenase (HLADH, E.C. 1.1.1.1), an enzyme with a wide substrate tolerance.[2] Our strategy for designing a series of inhibitors was based upon the reported, weak inhibitory effect of allyl alcohol[3] and, more significantly, upon the ability of HLADH to catalyse the *cis-trans*-isomerisation of farnesal.[4]

Both observations have been interpreted by invoking nucleophilic addition of an enzyme nucleophile to an αβ-unsaturated aldehyde, generated either by enzyme-catalysed oxidation[3] or present in the substrate,[4] according to Scheme 1.

Thus the allyl alcohol (1) affords the covalently inhibited enzyme (4) by protonation of the Michael adduct, whereas farnesal (3) undergoes reversible *cis-trans*-isomerisation, *via* the reverse mechanism to that of addition, giving (5). Allyl alcohol is a poor substrate for HLADH and we felt that the efficacy of this type of inhibitor might be improved if either the αβ-unsaturated aldehyde was made more electrophilic or if R^n in Scheme 1 became a leaving group that could compete with loss of the enzyme nucleophile (Scheme 2). This paper describes the preparation and properties of such compounds. Since this phase of our work was completed, Bright *et al.* have shown that the generation of allenes from suitable acetylenes, a well-tried strategy in the design of latent inhibitors, is effective with HLADH.[5]



SCHEME 1



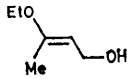
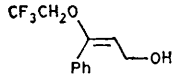
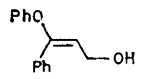
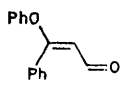
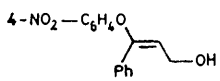
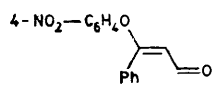
SCHEME 2

RESULTS AND DISCUSSION

A series of inhibitors covering a range of leaving group abilities was synthesised and tested against HLADH at pH 9, the optimum pH for alcohol oxidation. The structures and inhibitory properties of the compounds

TABLE 1

Inhibition properties of the compounds studied [a]

Compound	Structure	K_m/M	K_i/M	Inhibition properties
(1)	$CH_2=CHCH_2OH$	1.54×10^{-4}		Competitive, [b] slow inactivation
(6)	$HC\equiv C\cdot CH_2OH$	1.25×10^{-3}		Competitive; no time-dependent inactivation, forms malonic dialdehyde
(7)	$Cl_3CCH=CHCH_2OH$			Neither substrate nor inhibitor
(8)				No time-dependent inactivation
(9)				No time-dependent inactivation
(10)	$BrCH=CHCH_2OH$	6.4×10^{-4}		Competitive, no time-dependent inactivation, forms malonic dialdehyde
(11)	$EtSCH=CHCH_2OH$	5.0×10^{-6}		Rapid covalent inhibition at 10^{-5}M, $t_{\frac{1}{2}} = 2$ min
(12)	$EtSCH=CHCHO$		1.3×10^{-5}	Rapid covalent inhibition at 10^{-5}M, $t_{\frac{1}{2}} = 30$ s
(13)				No time-dependent inactivation
(14)			5.2×10^{-4}	Competitive, reversible
(15)				No time-dependent inactivation
(16)			5.2×10^{-4}	Competitive, reversible

[a] Compounds were assayed at pH 9 in the presence of NAD (1.5×10^{-3}M), using 3.75×10^{-7}—1.5×10^{-6}M HLADH. [b] 'Competitive' refers to competition with ethanol.

are summarised in Table 1. All were prepared by Michael addition of the appropriate leaving-group anion under base-catalysed conditions to either an αβ-unsaturated ester or an aldehyde. The trichloromethyl compound (7) was prepared *via* a Reformatsky reaction between chloral and ethyl bromoacetate dehydration of the intermediate alcohol, and reduction of the ester. The results show that only those compounds containing ethylthio-groups were potent inhibitors of HLADH.[6] Those with better leaving groups [(8)—(10) and (13)—(16)] were poor substrates, binding weakly and showing no evidence for time-dependent inactivation of the enzyme, even when presented in the reactive aldehyde form [(14) and (16)]. The trichloromethylallyl alcohol [7] (7), which should have generated a reactive, electrophilic inhibitor, failed to bind to the active site of HLADH. In view of the potency of the ethylthio-compounds (11) and (12), a more detailed kinetic study of their behaviour was undertaken.

Inhibition of HLADH by the Thioethers (11) *and* (12).—A true latent inhibitor must produce the inhibitor proper by the catalytic act of the enzyme in a reaction that is time-dependent and first order in both enzyme and substrate (latent inhibitor).

In the case of 3-ethylthioprop-2-en-1-ol (11), it was found that at 10^{-4}M, the half life of HLADH (10^{-7}M) was *ca.* 1 min (Figure 1). The requirement for the catalytic intervention of the enzyme was demonstrated initially by the fact that NAD^+ is essential for activation

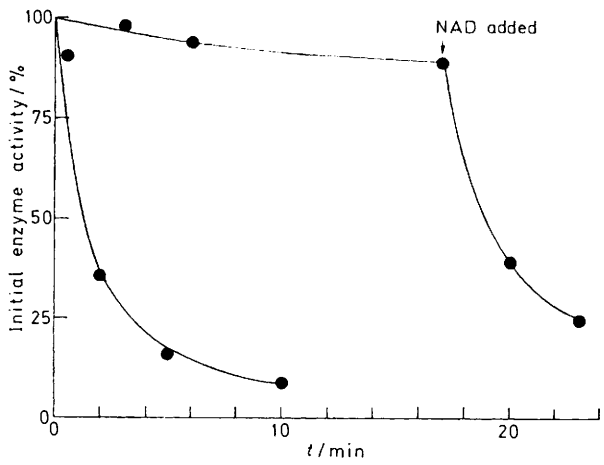


FIGURE 1 Inhibition of HLADH by (11). [(11)] 10^{-4}M, [HLADH] 10^{-7}M, [NAD] 5×10^{-4}M

(Figure 1). Further, it was shown that oxidation of 1,1-dideuterio-(11) was 1.4 times slower than that of the unlabelled compound and also that inhibition is correspondingly slower in the former case (Figure 2). Evidence for the kinetic order of the reaction is presented in Figure 3. Inhibition could be prevented by the addition of ethanol which competes with (11) for the active site of HLADH. A solvolysis product of (11) is not involved because preincubation of (11) with NAD^+ causes no initial inhibition of the enzyme.

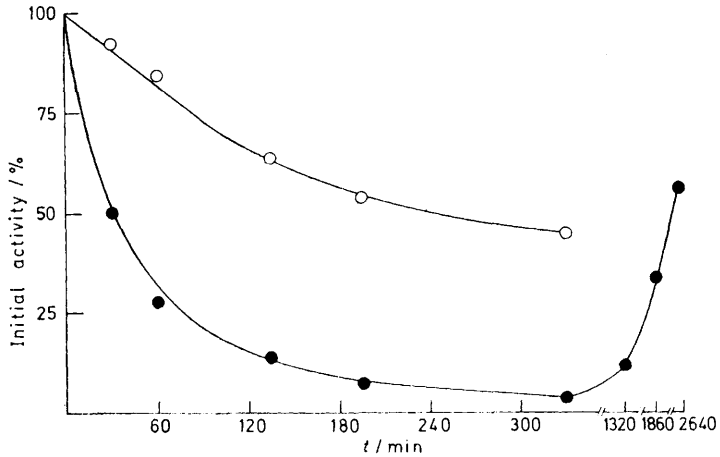


FIGURE 2 Inhibition of HLADH by (11) ● and [$^2H_2$]-(11) ○ and recovery of enzyme activity on standing. [(11)] 2×10^{-5}M, [HLADH] 10^{-7}M, [NAD] 5×10^{-5}M

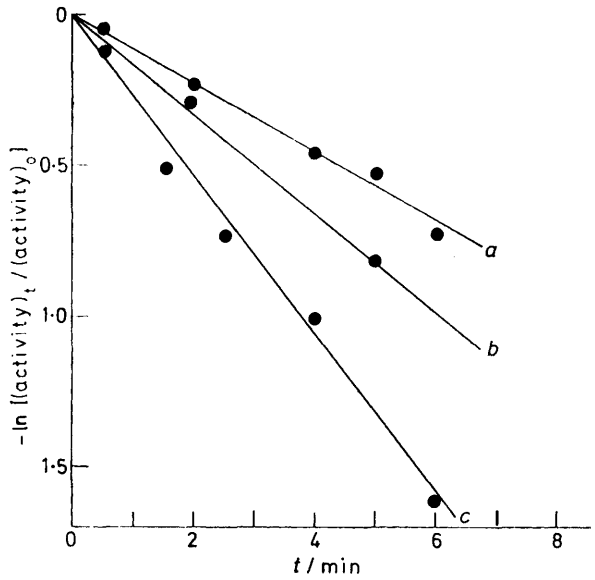
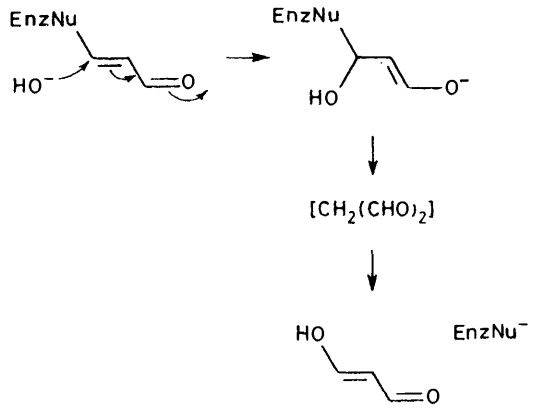


FIGURE 3 Concentration dependence of inhibition of HLADH by (11). [HLADH] 10^{-7}M, [NAD] 5×10^{-4}M; [(11)] *a* 5×10^{-4}M, *b* 10^{-5}M, *c* 2×10^{-5}M

Together, these results indicate that 3-ethylthioprop-2-en-1-ol is an effective latent inhibitor of HLADH and imply that the corresponding aldehyde (12) should also be an inhibitor. This was found to be the case; the aldehyde (12) reacts in a time-dependent manner analogous to the alcohol, but at a rate too fast for measurement by conventional, spectrophotometric techniques.

It is interesting to estimate the fraction of turnovers by the enzyme that gives rise to inactivation and the simplest estimate of this can be obtained by comparing the rate of oxidation with the rate of inactivation. In this way, the alcohol (11) was shown to be relatively efficient, about 9 out of 10 turnovers causing inactivation.

A limitation to the use of the ethylthioallyl derivatives became apparent when it was observed that, on prolonged standing for several days after inhibition by either the thioether (11) or (12), HLADH gradually

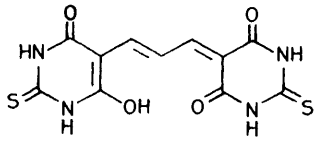


SCHEME 3

recovered activity (Figure 2). Recovery of enzyme activity could be due to hydrolysis of the covalently inhibited enzyme *via* the reverse process of the mechanism of inhibition in Scheme 2. The product of this reaction, in addition to the active enzyme, would be malonic dialdehyde (Scheme 3). In the absence of enzyme, both thioethers were stable under the conditions of assay as shown by u.v. spectroscopy.

The quantity of malonic dialdehyde can be estimated

using thiobarbituric acid,[8] two molecules of which condense with malonic dialdehyde to give an intense purple pigment with a maximum absorption at 530 nm. We isolated this pigment from a preparative-scale experiment using malonic dialdehyde acetal and were able to show that it has the structure (17). Using this



(17)

technique, it was shown that the rate of formation of malonic dialdehyde was, within experimental error, identical to the rate of formation of NADH when the alcohol (11) was oxidised by NAD^+ and HLADH (Figure 4). Hydrolysis of the enzyme inhibitor adduct

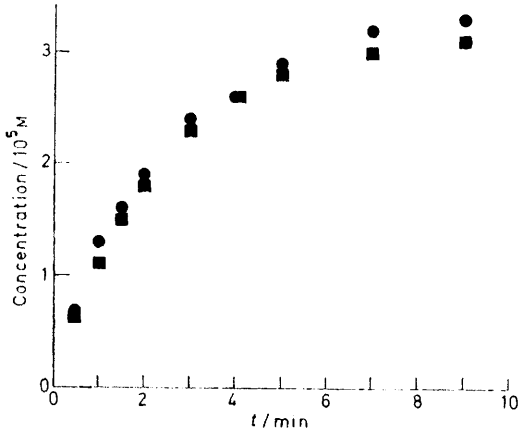


FIGURE 4 Products of oxidation of (11) by HLADH; ■ = NADH and ● = malonic dialdehyde

is thus surprisingly rapid and this was confirmed by the observation that almost all enzymic activity was recovered when inhibited enzyme was passed through Sephadex G25. Hydrolysis of covalently-bound inhibitors has been observed in other cases,[9,10] including Michael adducts.[11] The persistence of inhibition caused by the thioethers can, therefore, not be due to their covalent attachment to the enzyme's active site.

A solution to the paradox arose when it was pointed out to us that thiols, including ethanethiol, are competitive inhibitors of HLADH.[12] Although we were unable to quantify the presence of ethanethiol in solutions from inhibition experiments, it was clearly present from the smell. It was also possible to demonstrate that HLADH inhibited by ethanethiol gradually recovered activity on standing, as we had found using the thioethers (Table 2).

Malonic dialdehyde itself is a weak inhibitor of HLADH (35% inhibition at 1.76×10^{-3}M with 7.5×10^{-8}M HLADH and 1.5×10^{-3}M NAD^+ after addition of saturating ethanol). Significantly, it is produced enzymically from prop-2-ynol (6) and from the bromo-alcohol (10), as shown by thiobarbituric acid assay, as well as from the thioether (11). All three substrates are at the same oxidation state, and, after oxidation and Michael addition, could form the same enzyme-bound intermediate (Scheme 3). Rapid hydrolysis of this adduct affords malonic dialdehyde from all three and the persistent thiol inhibitor from only the thioether (11).

TABLE 2

Inhibition of HLADH by ethanethiol [a]

	Enzyme activity (% of control)	
Time/h	[EtSH] = 10^{-6}M	[EtSH] = 10^{-5}M
0	41.4	0.5
4	45.5	0.5
22	75.0	10

[a] [HLADH] 10^{-7}M, [NAD^+] 10^{-5}M, 0.1M pH 9 phosphate buffer.

Evaluation of the Inhibitors.—The above results indicate that, although the thioether (11) is a potent latent inhibitor of HLADH, the persistence of inhibition is due to release of ethanethiol at the active site. It is significant that of the compounds studied (Table 1), the thioethers bind most strongly to the active site of HLADH. This may reflect co-ordination of sulphur to the catalytic, zinc ion[13] and such bonding could also account for inhibition by ethanethiol. HLADH also contains a charge relay system at the active site. Hydrolysis of electrophilic groups bonded at the active site can be understood in this way. Indeed, the parallel formation of malonic dialdehyde and NADH renders the involvement of a covalently bonded enzyme-inhibitor complex unessential: enzyme–catalysed hydrolysis of the aldehyde (12) *via* Michael addition could lead directly to the persistent inhibitor, ethanethiol. Nevertheless, latent inhibition *via* Michael addition reactions is clearly successful with HLADH and may be more controllable in the absence of complicating thiol groups.

EXPERIMENTAL

^{1}H N.m.r. spectra were recorded on a Perkin-Elmer R12 spectrometer operating at 100 MHz, except where otherwise stated; chemical shifts are on the δ scale. Horse liver alcohol dehydrogenase (Böhringer, Sigma) was assayed in 0.1M potassium phosphate buffer, pH 9 unless otherwise stated, at 30 °C, following the method of Blair and Vallee.[14] Kinetic constants were obtained using the graphical methods of Dixon [15] and Cornish-Bowden and Eisenthal.[16,17] Time-dependent inhibition experiments were performed by removing samples from the bulk reaction and quenching inhibition rapidly with ethanol (10^{-2}M) immediately before measuring the absorbance.

Ethyl 4,4,4-Trichlorobutenoate.—Ethyl bromoacetate (34.6 g), freshly distilled chloral (29.4 g), and zinc dust (14.6 g) were covered with benzene (40 ml) and ether (40 ml). The mixture was gently warmed with stirring to initiate the reaction; cooling was subsequently applied to maintain gentle reflux. When the reaction subsided, zinc dust (1 g) was added and the mixture heated under reflux until ethyl bromoacetate could no longer be detected by smell. The mixture was cooled and then poured into ice (50 g) and concentrated sulphuric acid (5 ml). The acidic layer was separated and washed with ether (2 × 10 ml). The com-

bined ethereal layers were washed with saturated aqueous sodium hydrogencarbonate and water, and dried ($CaCl_2$). Evaporation of the solvent afforded the crude, intermediate hydroxy-ester (42 g).

The hydroxy-ester (35 g) was added to phosphorus pentaoxide (14 g) and the mixture stirred, at 50 °C, for 2 h. Distillation under reduced pressure afforded the required ester (12 g), b.p. 102–106 °C/25 mmHg. ^{1}H N.m.r. spectroscopy indicated that a mixture of the *cis*- and *trans*-isomers (5:1) was obtained δ (60 MHz, $CDCl_3$) 1.32 (3 H, overlapping t), 4.27 (2 H, overlapping q), 6.24, 6.50 and 7.11, 7.37 (2 H, AB quartet, *J* 16 Hz *trans*-isomer), 6.60, and 6.90 (*J* 12 Hz, *cis*-isomer).

(E)-4,4,4-*Trichlorobut*-2-*en*-1-*ol* (7).—Ethyl 4,4,4-trichlorobutenoate (10.85 g) was dissolved in dry ether (160 ml). A slurry of lithium aluminium hydride (0.38 g) and aluminium trichloride (13.3 g) in ether was added with cooling and vigorous stirring during 0.5 h, the temperature being maintained between 15 and 20 °C. After the addition was complete, stirring was continued for 0.5 h and then aqueous methanol (50%, 20 ml) was added during 0.5 h followed by 6M sulphuric acid (50 ml). The mixture was extracted with ether, the ether layer washed with saturated, aqueous sodium hydrogencarbonate, and dried ($MgSO_4$). Evaporation under reduced pressure afforded an oil (9 g) consisting of the required alcohol and some unchanged ester. The mixture was readily separated by column chromatography on silica gel, the ester being eluted with light petroleum –chloroform (2 : 3 v/v) and the alcohol with chloroform. The latter had b.p. 98—102 °C/20 mmHg (lit.,[7] 102—103 °C/17 mmHg); ^{1}H n.m.r. ($CDCl_3$, 60 MHz) δ 4.00 (2 H, d, *J* 6 Hz), 4.95 (1 H, m), and 6.25 (1 H, d, *J* 11 Hz).

Ethyl 3-*Ethoxybut*-2-*enoate*.—This compound was prepared following the literature procedure.[18]

(E)-3-*Ethoxybut*-2-*en*-1-*ol* (8).—The alcohol (8) was prepared from the above ester in the same way as the alcohol (11) below. The required *alcohol* (75% yield) had b.p. 110—112 °C/50 mmHg (Found: C, 62.0; H, 10.6. $C_6H_{12}O_2$ requires C, 62.1; H, 10.3%); ^{1}H n.m.r. ($CDCl_3$) δ 1.24 (3 H, t, *J* 7 Hz), 1.80 (3 H, s), 2.27 [1 H, s (exchanges with D_2O)], 3.61 (2 H, q, *J* 7 Hz), 4.00 (2 H, d, *J* 8 Hz), and 4.57 (1 H, t, *J* 8 Hz).

Ethyl 3-*Bromopropenoate*.—Ethyl propiolate (4 g) was cooled, with stirring, in an ice-bath and a solution of hydrogen bromide in acetic acid (48% w/v, 7.32 ml) was added in drops during 5 min. The solution was allowed to warm to room temperature and was stirred for 1 h. Evaporation of the acetic acid under reduced pressure afforded an oil which was distilled. The fraction boiling between 60 and 94 °C/45 mmHg was collected. This oil was dissolved in ether and the solution washed twice with saturated, aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and evaporated to dryness. The residual oil was redistilled, affording the required ester (4.8 g), b.p. 94—97 °C/55 mmHg. The product was an approximately 3 : 2 mixture of the (*Z*)- and(*E*)-isomers; ^{1}H n.m.r. ($CDCl_3$, 60 MHz) δ 1.26 (3 H, dt), 4.20 (2 H, dq), 4.60, 6.99 [d, *J* 8 Hz, (*Z*)-isomer], 6.52, and 7.70 (d, *J* 14 Hz, (*E*)-isomer].

(E)-*and* (Z)-3-*Bromoprop*-2-*en*-1-*ol* (10).—To a solution of ethyl bromopropenoate in dry ether (30 ml), lithium aluminium hydride (0.3 g) was added with stirring and cooling so that the temperature remained less than 5 °C. After the addition was complete, the mixture was stirred for a further 2 h at room temperature. Excess of reducing agent was then destroyed with ethyl acetate and saturated aqueous sodium sulphate. The precipitate was filtered off and washed with ether, and the ether layer was dried (Na_2SO_4) and evaporated. The residual oil was purified by chromatography on silica gel, the unchanged ester being eluted with petroleum (b.p. 40—60 °C) and the required alcohol with ether. Redistillation afforded a colourless oil, b.p. 70 °C/20 mmHg (lit.,[19] *cis*, 81 °C/25 mmHg, *trans*, 87 °C/25 mmHg) (Found: C, 26.3; H, 3.8. C_3H_5BrO requires C, 26.3; H, 3.65%); ^{1}H n.m.r. ($CDCl_3$) δ 2.28 (1 H, s), 4.21 (2 H, d), and 6.18 (3 H, m).

(E)-3-*Phenyl*-3-(2,2,2-*trifluoroethoxy*)*propenal*.—3-Phenylpropynal[20] (5 g) was dissolved in dry ether and pyridine (1 ml) added. Trifluoroethanol (3.8 g) was added in drops, with stirring; the solution was stirred at room temperature for 3 d. Evaporation of the solvent left a black oil which was distilled using a kugelrohr apparatus affording the required *aldehyde* (4.1 g, 47%), b.p. 200 °C/0.05 mmHg. The distillate was three times recrystallised from light petroleum to yield colourless crystals, m.p. 72—74 °C [Found: *m/e* 230.0588 (M^+). $C_{11}H_9F_3O_2$ requires 230.0633].

(E)-3-*Phenyl*-3-(2,2,2-*trifluoroethyl*)*prop*-2-*en*-1-*ol* (9).—The above aldehyde (1.0 g) was dissolved in ethanol (50 ml) and sodium borohydride (0.3 g) added in portions to the stirred solution at room temperature. Stirring was continued for 2 h and aqueous sodium hydroxide (40 ml; 2M) added. The product was extracted with dichloromethane (2 × 100 ml) and the organic solution dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded a colourless oil (0.97 g). A portion was redistilled using a kugelrohr apparatus to give the *alcohol* (9), b.p. 180 °C/0.05 mmHg [Found: C, 56.9; H, 4.8%; *m/e* 232.070 (M^+). $C_{11}H_{11}F_3O_2$ requires C, 56.8; H, 4.74%; *M*, 232.0711]; ^{1}H n.m.r. ($CDCl_3$) δ 1.9 [1 H, s (exchanges with D_2O)], 4.05, (2 H, q, *J* 8 Hz), 4.05 (2 H, d *J* 8 Hz), 4.96 (1 H, t *J* 8 Hz), and 7.24 (5 H, s).

Ethyl 3-*Ethylthiopropenoate*.—To a solution of ethyl propiolate (20 g, 0.204 mol) in dry ether (100 ml), dry triethylamine (10 ml) was added. Ethanethiol (15 ml) was added in drops, with stirring, and a vigorous reaction ensued. The solution was set aside for 1.5 h after which the solvent was removed under reduced pressure; the residue was then redistilled, also under reduced pressure, to afford the required *ester* (28 g, 86%), b.p. 120—125 °C/10 mmHg (Found: C,51.4; H, 8.8. $C_7H_{12}O_2S$ requires C, 51.9; H, 8.5%).

(E)-3-*Ethylthioprop*-2-*en*-1-*ol* (11).—The ester (12) (20 g) was dissolved in dry ether (300 ml) and lithium aluminium hydride (40 g) was added in portions, with stirring, during 1 h at room temperature. The mixture was set aside overnight after which excess of lithium aluminium hydride was destroyed with ethyl acetate and saturated, aqueous sodium sulphate. The ether layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure; the residue was then redistilled, also under reduced pressure, to give the required alcohol (8.2 g, 56%), b.p. 68—70 °C/0.5 mmHg (lit.,[21] 73 °C/0.6 mmHg) (Found: C,51.0; H, 8.6; S, 26.4. $C_5H_{10}OS$ requires C, 50.9; H, 8.47; S, 27.1%); ^{1}H n.m.r. ($CDCl_3$) δ 1.27 (3 H, t, *J* 7 Hz), 2.64 (2 H, q, *J* 7 Hz), 2.00 [1 H, s (exchanges with D_2O)], 4.02 (2 H, d, *J* 6 Hz), 5.59 (1 H, dt, *J* 6 and 15 Hz), and 6.14 (1 H, d, *J* 15 Hz).

Similarly prepared was the 1,1-dideuterio-alcohol using lithium aluminium deuteride. The ^{1}H n.m.r. spectrum was identical with that of (11) except that the 4.02 CH_2 reson-

ance was absent and the 5.59 resonance had collapsed to a doublet.

(E)-3-*Ethylthiopropenal* (12).—Propiolaldehyde was prepared from prop-2-ynol [22] (56 g) and the aldehyde distilled directly into a cold trap (−78 °C) containing ethanethiol (62 g) dissolved in dry ether (100 ml) to which sodium ethoxide in ethanol (1M, 2 drops) was added. After production of the aldehyde was complete, the contents of the cold trap were allowed to warm to room temperature and to stand overnight. The solvent and excess ethanethiol were then removed at −5 °C under reduced pressure, to leave a residual oil (24 g) the distillation of which at 4 mmHg evolved ethanethiol and afforded an oil, b.p. 116–120 °C (15 g). The oil was characterised, by ^{1}H n.m.r., as 2,3-diethylthiopropanal.

2,3-Diethylthiopropanal (1.0 g) was dissolved in chloroform (20 ml) and mercuric oxide (1.4 g) added. The mixture was heated for 8 h under reflux and a grey precipitate of mercury formed. The solution was decanted, the solvent removed under reduced pressure, and the residue redistilled affording the required *aldehyde*, b.p. 60 °C/0.5 mmHg, (0.3 g, 46%); ^{1}H n.m.r. (60 MHz, $CDCl_3$) δ 1.39 (3 H, t, *J* 7 Hz), 2.93 (2 H, q, *J* 7 Hz), 6.18 (1 H, dd, *J* 7 and 15 Hz), 7.72 (1 H, d, *J* 15 Hz), and 9.55 (1 H, d, *J* 7 Hz). The semicarbazone was prepared, m.p. 147 °C (Found: C, 41.2; H, 7.3; N, 17.8; S, 27.3. $C_6H_{11}N_3OS$ requires C, 40.9; H, 7.2; N, 17.9; S, 27.2%).

(E)-3-*Phenoxy*-3-*phenylpropenal* (14).[23]—Phenylpropynal (0.6 g) was dissolved in absolute ethanol (1.5 ml) containing pyridine (0.1 ml). To this solution was added a solution of phenol (0.47 g) in absolute ethanol (5 ml) and the mixture heated under reflux for 1 h. After evaporation of the solvent under reduced pressure, the solid residue was recrystallised from light petroleum affording the required *aldehyde* (0.40 g), m.p. 88 °C (Found: C, 80.6; H, 5.7. $C_{15}H_{12}O_2$ requires C, 80.4; H, 5.36%); ^{1}H n.m.r. ($CDCl_3$) δ 5.36 (1 H, d, *J* 9 Hz), 6.95—7.70 (10 H, m), and 9.35 (1 H, d, *J* 9 Hz).

2-(4-*Nitrophenoxy*)-3-*phenylpropenal* (16).—This compound was prepared in a manner identical to that described above from *p*-nitrophenol. The *aldehyde* (0.65 g) had m.p. 103—104 °C (from light petroleum–CCl_4) [Found: C, 66.7; H, 4.0; N, 4.9%; *m/e* 269.0688 (M^+). $C_{15}H_{11}NO_4$ requires C, 66.7; H, 4.09; N, 5. 20%; *M*, 269.0688]; ^{1}H n.m.r. ($CDCl_3$) δ 5.50 (1 H, d, *J* 8 Hz), 7.13 (2 H, d, *J* 9 Hz), 7.42 (5 H, m), 8.15 (2 H, d, *J* 9 Hz), and 9.42 (1 H, d, *J* 8 Hz).

(E)-3-*Phenoxy*-3-*phenylprop*-2-*en*-1-*ol* (13).—The same preparation as used for the trifluoroethyl analogue (9) gave the required *alcohol* (85%), b.p. 180 °C/0.05 mmHg (kugelrohr) (Found: C, 79.1; H, 6.3%. $C_{15}H_{14}O_2$ requires C, 79.6, H, 6.2%); ^{1}H n.m.r. ($CDCl_3$) δ 4.14 (2 H, d, *J* 9 Hz), 5.25 (1 H, t, *J* 9 Hz), and 6,80—7.43 (10 H, m).

(E)-3-(4-*Nitrophenoxy*)-3-*phenylprop*-2-*en*-1-*ol* (15).—The same preparation as used for the trifluoroethyl analogue (9) gave the required *alcohol* (87%), m.p. 98—99 °C (from light petroleum) [Found: C, 66.1; H, 4.8; N, 5.4%; *m/e* 271.0843 (M^+). $C_{15}H_{13}NO_4$ requires C, 66.4; H, 4.8; N, 5.2%; *M*, 271.0845], ^{1}H n.m.r. ($CDCl_3$) δ 2.09 [1 H, s, (exchanges with D_2O)], 4.25 (2 H, d, *J* 9 Hz), 5.64 (1 H, t, *J* 9 Hz), 68.6 (2 H, d, *J* 9 Hz), 7.25 (5 H, m), and 7.93 (2 H, d, *J* 9 Hz).

5-[3-(6-*Hydroxy*-4-*oxo*-2-*thioxo*-1,2,3,4-*tetrahydropyrimidin*-5-*yl*)*prop*-2-*enylidene*]-2-*thioxo*-2,3-*dihydropyrimidine*-4,6(1H,5H)-*dione* (17).—Thiobarbituric acid (0.9 g) and 1,1,3,3-tetraethoxypropane (0.68 g) were dissolved in 12% hydrochloric acid and heated under reflux for 1½ h. A deep-purple precipitate formed which was filtered off after cooling of the solution and washed with 0.6M hydrochloric acid (50 ml), hot water, ethanol (10 ml), ethanol–ether (1 : 1 v/v, 50 ml), and ether (50 ml). After drying under reduced pressure the product (0.9 g) was obtained in 93% yield, m.p. >330 °C (Found: C, 38.9; H, 3.0; N, 16.1; S, 18.6. $C_{11}H_8N_4O_4S_2\cdot 2H_2O$ requires C, 38.6; H, 2.9; N, 16.4; S, 18.4%); ^{1}H n.m.r. (D_2O–NaOD) δ 7.80 (2 H, d) and 8.50 (1 H, dt); ^{13}C n.m.r. (D_2O–NaOD) δ 106.42, 120.79, 157.92, 175.33, and 198.57 p.p.m. (w.r.t. tetramethylsilane).

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