Synthesis of (*R*)-[1-^{2}H,1-^{3}H]- and [1,1,10,10-$^2H_4$]-Decane: Inverse Isotope Effects in the Protonation of Anions from 1,3-Dithians

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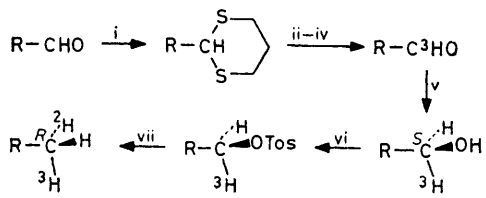
A protocol for applying substrates with chiral methyl groups to determine the stereochemistry of enzymic hydroxylation of methyl groups is discussed. Efficient methods are described for the synthesis of (*R*)-[1-^{2}H,1-^{3}H]- and [1,1,10,10-$^2H_4$]-decane from decanal and diethyl sebacate, respectively. Decanal was converted *via* the 2-anion of 2-nonyl-1,3-dithian to [1-^{3}H]decanal which was reduced to (*S*)-[1-^{3}H]decan-1-ol by NADH–liver alcohol dehydrogenase. Tosylation of the decanol, followed by reduction with Li[Al$^2H_4$] gave (*R*)-[1-^{2}H,1-^{3}H]-decane (specific activity 0.095 Ci mol^{-1}). Diethyl sebacate was reduced with Li[Al$^2H_4$] to [1,1,10,10-$^2H_4$]decane-1,10-diol. Conversion of the diol to its ditosylate and reduction with Li[AlH_4] gave [1,1,10,10-$^2H_4$]-decane. Although these procedures are essentially adaptations of literature methods, several significant improvements are described. The protonation (deuteriation, tritiation) of the 2-anion of 2-nonyl-1,3-dithian shows an inverse isotope effect, the magnitude of which has been explored for 2-anions of several 2-alkyl- and 2-aryl-1,3-dithianes (see Table).

In the degradation of n-alkanes by liver microsomes or certain micro-organisms[1] the first step is enzymic ω-oxidation to a primary alcohol. This process is remarkable for its regiospecificity. It is potentially of value for producing commercially useful primary alcohols. The enzymatic system ('mono-oxygenase') responsible for the oxidation of n-alkanes to primary alcohols has been purified in some cases.[2,3] One type of mono-oxygenase, *e.g.* that of liver microsomes, consists of the hemoprotein cytochrome P-450, an NADPH-dependent cytochrome P-450 reductase, and phosphatidylcholine.[2] A second type depends on non-heme ion, *e.g.* the mono-oxygenase from *Pseudomonas oleovorans* has been resolved into three components, a non-heme iron protein ('ω-hydroxylase'), rubredoxin, and NADH-dependent rubredoxin reductase.[3]

The mechanism of action of oxygenases in general is poorly understood, the nature of the oxidising agent derived from dioxygen having not been identified.[4,5] In order to contribute to an understanding of the mechanistic problem, we have synthesised n-decane containing a chiral methyl group {(*R*)-[1-^{2}H,1-^{3}H]-decane}. The method we have developed should be applicable to a variety of alkanes and other substrates for mono-oxygenases acting on methyl groups. Decane was chosen as initial target molecule because of its ease of handling and its property as a substrate for several micro-organisms.[1] Enzymic ω-oxidation of [1-^{2}H,1-^{3}H]-n-decane will give the enantiomers of [1-^{2}H,1-^{3}H]-, [1-^{2}H]-, and [1-^{3}H]-decan-1-ol in proportions which will depend on the primary kinetic isotope effect for the oxidative step and its stereochemistry. If a normal isotope effect prevails (*i.e.* $k_H > k_D > k_T$) the principal product will be [1-^{2}H,1-^{3}H]decan-1-ol. If (*R*)-[1-^{2}H,1-^{3}H]decane is used as substrate, replacement of H at C-1 with retention of configuration would give (*S*)-[1-^{2}H,1-^{3}H]decan-1-ol. This result might obtain if the nature of the oxidising agent is electrophilic. For all of several cases studied, enzymic oxidation of a CH_2 group to CHOH proceeds with retention of configuration.[6] The possibility that enzymic oxidation of alkanes may go *via* hydroxyl radicals has been raised.[7] In this case, alkyl radicals might be intermediates and so a racemic mixture of (*R*)- and (*S*)-[1-^{2}H,1-^{3}H]decan-1-ol could be formed. Therefore, a study of the stereochemistry of enzymic ω-oxidation of (*R*)-[1-^{2}H,1-^{3}H]-decane is likely to throw light on mechanism. This substrate will permit different mono-oxygenases to be characterised on a mechanistic basis and should aid the characterisation of chemical model systems.[4,8] Non-enzymic methods for selectively oxidising alkanes to primary alcohols have not yet been developed.



Scheme Synthesis of (*R*)-[1-^{2}H,1-^{3}H]decane from decanal (R = n-C_9H_{19}). Reagents: i, propane-1,3-dithiol-HCl; ii, BuLi; iii, ^3H_2O; iv, hydrolysis; v, LADH–NADH; vi, TsCl–pyridine vii, Li[Al$^2H_4$]

Synthesis of (R)-[1-^{2}H,1-^{3}H]*Decane*.—Our synthesis of decane with a chiral methyl group (*cf.* Scheme) resembles part of the route used by Arigoni and Luthy[9] for the preparation of chiral acetic acid. They reduced [2-^{3}H]-glyoxylic acid enzymically to (*S*)-[2-^{3}H]glycolic acid, which was esterified and reduced with Li[AlH_4] to

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[2] D. A. Haugen and M. J. Coon, *J. Biol. Chem.*, 1976, **251**, 7929.
[3] T. Ueda and M. J. Coon, *J. Biol. Chem.*, 1972, **247**, 5010.
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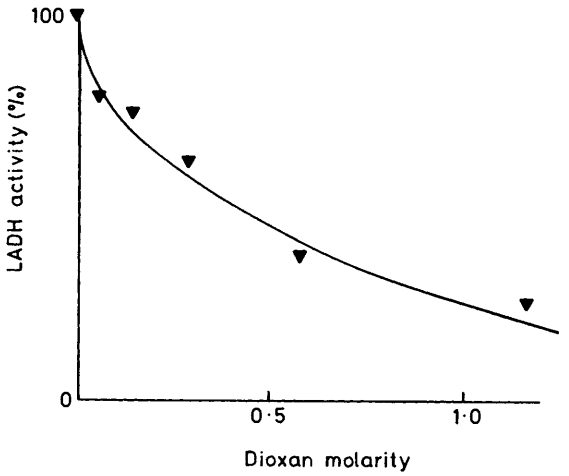
[5] For a recent discussion see S. W. May, S. L. Gordon, and M. S. Steltenkamp, *J. Amer. Chem. Soc.*, 1977, **99**, 2017.
[6] See *e.g.* A. R. Battersby, P. W. Sheldrake, J. Staunton, and D. C. Williams, *J.C.S. Chem. Comm.*, 1974, 566.
[7] G. V. Buxton, J. C. Green, R. Higgins, and S. Kanjii, *J.C.S. Chem. Comm.*, 1976, 158.
[8] See *e.g.* J. T. Groves and M. Van Der Puy, *J. Amer. Chem. Soc.*, 1976, **98**, 5290.
[9] (*a*) J. Lüthy, J. Rétey, and D. Arigoni, *Nature*, 1969, **221**, 1213; J. Lüthy, Diss. No. 4764, E. T. H. Zurich, 1972; (*b*) J. W. Cornforth, *Tetrahedron*, 1974, **30**, 1515.

(*S*)-[1-^{3}H]ethane-1,2-diol. The diol was converted to its mono-*p*-bromobenzenesulphonate which was reduced with Li[Al^{2}H$_4$] to a mixture of (2*R*)-[2-^{2}H,2-^{3}H]- and (1*S*)-[2-^{2}H,1-^{3}H]-ethanol. Oxidation of this mixture gave acetic acid and (*R*)-[2-^{2}H,2-^{3}H]acetic acid.

The starting material, [1-^{3}H]decanal, for our synthesis of (*R*)-[1-^{2}H,1-^{3}H]decane, was prepared from decanal by modifying the procedure of Seebach *et al.*[10] 2-Nonyl-1,3-dithian (1a) in tetrahydrofuran (THF) was converted to its bis(thio)carbanion (2a) by butyl-lithium–hexane (7 h and −5 to 0°). Efficient generation of (2a) under these conditions was checked by quenching with ^2H_2O and assaying the resulting [2-^{2}H]- (1a) by ^{1}H n.m.r. spectroscopy (>99% deuteriation at C-2). The reaction between (1a) and butyl-lithium at −60° (conditions of ref. 10) was incomplete after several hours (30% deuteriation at C-2). The efficiency of tritiation when (2a) is treated with tritiated water will be governed by their molar ratios and the isotope effect associated with proton (triton) transfer. To assess the outcome of tritiation we studied the competition of (2a) for protons *versus* deuterons by quenching with 1 : 1 water–^2H_2O. This shows (see later section) that protonation of (2a) is associated with an inverse isotope effect and therefore a substantial proportion of ^{3}H in a sample of tritiated water will react with (2a). Using the conditions described in the Experimental section we obtained [1-^{3}H]-(1a) of specific activity 35 Ci mol^{-1}. The very high activity of [1-^{3}H]-(1a) permits a dilution at a subsequent stage. This material was hydrolysed to [1-^{3}H]decanal *via* 1,1-dimethoxy[1-^{3}H]decane. Analogously, we have prepared [1-^{3}H]octanal from octanal *via* (1b).

Reduction of [1-^{3}H]decanal to (*S*)-[1-^{3}H]decan-1-ol was accomplished using horse liver alcohol dehydrogenase (LADH) with nicotinamide adenine dinucleotide (NADH). At the outset of our work it was not known whether decanal was a substrate for LADH, although it had been shown[11] that decan-1-ol is a substrate for yeast alcohol dehydrogenase. We found that 5μM-decanal reacts at a satisfactory rate. An additional problem to face in preparative scale reductions was the very low solubility of decanal in water [extrapolated value (*cf.* refs. 12 and 13) 1.3×10^{-5} mol dm^{-3} at 293 K]. To try to increase the solubility, we examined solutions of decane (0.03 g cm^{-3}) in a series of water-miscible solvents (acetonitrile, t-butyl alcohol, diethylene glycol, dimethyl ether, dimethylformamide, dimethyl sulphoxide, dioxan, and ethanol). When a drop of these solutions was added to varying amounts of phosphate buffer (1—3 cm^3) decanal separated in each case. Furthermore, each solvent inhibited LADH to a certain extent. Detergents [sodium dodecyl sulphate, Triton X-100, hexadecyltrimethylammonium bromide (Cetab), and lysolecithin] were also tried as solubilising agents but were inhibitory towards LADH and not very effective as solubilisers at concentrations where their inhibitory action was low. Finally, we selected dioxan as a solubilising agent because it was able to increase the solubility of decanal in phosphate buffer *ca.* 25-fold at a concentration (89mM-dioxan) where its inhibitory effect was low (see Figure). Graves *et al.*[14] used dioxan as solubiliser in the reduction of a series of alkylcyclohexanones by LADH. The reduction of [1-^{3}H]decanal (0.1 mmol) by LADH–NADH in phosphate buffer took place smoothly. To facilitate recovery of the [1-^{3}H]-decan-1-ol and execution of the final stages of the synthesis, it was diluted with unlabelled decan-1-ol (3.4 g) to a specific activity of 0.126 Ci mol^{-1}.

[1-^{3}H]Decan-1-ol obtained by LADH reduction of decanal is expected[15] to have the (*S*)-configuration.



Effect of added dioxan on LADH activity in the reduction of acetaldehyde (1.00mM) with 2.45mM-LADH and 0.182mM-NADH in phosphate buffer (pH 7; 2.00 cm^3)

' If a given enzyme can catalyse the oxidation–reduction of a variety of substrates, the stereospecificity of the hydrogen transfer will be the same for all the substrates.'[15] That LADH reduces [1-^{2}H]aldehydes to (*S*)-[1-^{2}H]alcohols has been established in a number of cases.[16] Application of [1-^{2}H,1-^{3}H]decane for determining the stereochemistry of enzymatic ω-oxidation does not require knowledge of the absolute configuration of [1-^{3}H]decan-1-ol, because the chirality of the alcohol formed by ω-oxidation can best be determined by LADH (*i.e.* by the reverse of one of the reactions used in the synthesis of [1-^{2}H,1-^{3}H]decane).

(*S*)-[1-^{3}H]Decan-1-ol was converted to its tosylate, which was reduced to (*R*)-[1-^{2}H,1-^{3}H]decane by Li[Al^{2}H$_4$] in ether. The analogous reduction of hexadecyl tosylate[17]

[10] D. Seebach, B. W. Erickson, and G. Singh, *J. Org. Chem.*, 1966, **31**, 4303.
[11] J. van Eys and N. O. Kaplan, *J. Amer. Chem. Soc.*, 1957, **79**, 2782.
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[13] G. Saracco and E. S. Marchetti, *Ann. Chim. (Italy)*, 1958, **48**, 1357.
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[16] H. S. Mosher, *Tetrahedron*, 1974, **30**, 1733; A. R. Battersby, J. Staunton, and H. R. Wiltshire, *J.C.S. Perkin I*, 1975, 1156.
[17] J. Strating and H. J. Backer, *Rec. Trav. chim.*, 1950, **69**, 638.

(0.25M in ether) by 0.25M-Li[AlH_4] is described [18] as instantaneous.' We find that reduction of decyl tosylate (0.1M in ether) by 0.1M-Li[AlH_4] has progressed only 25% after 4 h reflux and 50% after 32 h reflux.

Compound	Conditions [a]	Recovery (%)	Determinations per run (including control)	Kinetic isotope effect k_H/k_D ± average error [c]
(1a)	A	64	3	0.73 ± 0.07 [d]
	A	94	3	0.975 ± 0.005
	B	*b*	3	
(1b)	A	90	3	0.85 ± 0.07
(1c)	A	Not determined	3	0.83 ± 0.07 [d]
(1d)	A	90	3	1.14 ± 0.31
	B	*b*	3	
(1e)	A	Not determined	3	0.72 ± 0.06
(1f)	A	Not determined	3	0.68 ± 0.07
(1g)	A	71	3	0.88 ± 0.02
	B	55	3	1.65 ± 0.13
(1h)	A	*b*	4	

[a] A THF–n-hexane (2 : 1—3.3 : 1 v/v); B THF–HMPA–n-hexane (1 : 1 : 1 v/v). [b] Destroyed (these runs developed a very strong unpleasant smell). [c] k_H/k_D is the average mean value. Average error = Σ (*X*$_i$ — arithmetic mean)/number of measurements. [d] Corrected from the control.

For efficient reductions of alcohol tosylates to the corresponding alkane we use treatment with Li[AlH_4] or Li[Al^2H_4] in refluxing ether for ≥100 h (0.05M concentrations of tosylate and hydride). The period of refluxing required can be shortened to *ca.* 50 h using THF as solvent, but this incurred subsequent losses of decane when the THF was removed (rotary evaporator). We also tried Na[BH_4] in dimethyl sulphoxide [19] for the reduction of decyl tosylate to decane. This reaction was very sensitive to traces of moisture, decan-1-ol being produced as a by-product in amount proportional to the water present. The best yield of decane was only *ca.* 50%. The reduction of (*S*)-[1-^{3}H]decyl tosylate was therefore carried out using Li[Al^2H_4] in refluxing ether giving pure [1-^{2}H,1-^{3}H]decane in 81% yield. The reduction of alcohol tosylates by Li[AlH_4] occurs with inversion of configuration.[20] Hence, the configuration of our [1-^{2}H,1-^{3}H]decane is *R*. In an attempt rigorously to prove this assignment, we tried, in model experiments, to chlorinate decane selectively at an ω-1 position, intending to convert 2-chlorodecane to decan-2-ol, decan-2-one, and eventually acetic acid. Application of such a sequence to [1-^{2}H,1-^{3}H]decane would yield a sample of [2-^{2}H,2-^{3}H]acetic acid, the configuration of which could be determined by Cornforth's method.[9] Minisci and his co-workers [21] have reported chlorination of heptane to a mixture of chloroheptanes (mainly the 2-isomer) by *N*-chlorodimethylamine or *N*-chlorodi-isopropylamine–Fe^{II}–trifluoroacetic acid. However, in our hands, numerous 'reactions' between heptane or decane and *N*-chlorodi-isopropylamine (prepared in two ways), iron(II) sulphate, and sulphuric acid (sometimes with acetic or trifluoroacetic acid), either following the literature directions or modified procedures, failed to yield detectable chloroalkane (analysis by g.l.c. and ^{1}H n.m.r.). Finally, attempts to hydroxylate decane using triethylamine *N*-oxide–Fe^{II}–trifluoroacetic acid [22] gave at best traces of decanols, after basic hydrolysis of the crude product (supposedly containing trifluoroacetates).*

Synthesis of [1,1,10,10-$^2H_4$]*Decane.*—This compound was prepared as a substrate for mono-oxygenases, to test that ω-oxidation takes place with a normal isotope effect. It was prepared by reducing diethyl sebacate with Li[Al^2H_4], tosylating the resulting diol, and reducing the ditosylate with Li[AlH_4]. This sequence is similar to procedures used to prepare [1,1,1,8,8,8-$^2H_6$]octane [23] and [1,1,1,12,12,12-$^2H_6$]dodecane.[24] However, in these cases very low yields were obtained in the final stage (*e.g.* 14.5% in ref. 23) probably because the reaction times were too short. By using a reflux time of 200 h we obtained 77% pure [$^2H_4$]decane. Again, Na[BH_4] in dimethyl sulphoxide (⟶ *ca.* 30% decane) and Li[AlH_4] in THF (⟶ *ca.* 60% decane) were inadequate reducing agents.

Isotope Effects in the Protonation of Bis(thio)carbanions from 1,3-*Dithians.*—Eliel and his co-workers [25] have reported that k_H/k_D for deprotonation of 1,3-dithian is 2.5 ± 0.1. This value was obtained for the reaction between [2-^{2}H]-1,3-dithian and butyl-lithium in HMPA–THF–hexane (1 : 1 : 1). We have measured isotope effects for competitive quenching (H_2O *versus* HO^{2}H *versus* ^2H_2O) of the carbanions (2a—g), derived from a series of 2-alkyl- and 2-aryl-1,3-dithians (1a—g). Results are given in the Table. The carbanions were generated in THF–hexane or HMPA–THF–hexane. That formation of carbanion was complete under the conditions of each experiment, was checked by treating

* N. C. Deno, personal communication, has suggested modifications which should ensure efficient ω-1-chlorination or hydroxylation of decane.

[18] N. G. Gaylord, 'Reduction with Complex Metal Hydrides,' Interscience, New York, 1956, p. 855.
[19] R. O. Hutchins, D. Hoke, J. Keogh, and D. Koharski, *Tetrahedron Letters*, 1969, 3495.
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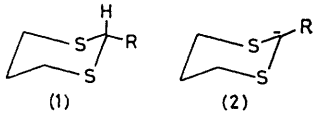
[24] G. J. Shaw and G. W. Milne, *J. Labelled Compounds*, 1976, **12**, 557.
[25] E. L. Eliel, *Tetrahedron*, 1974, **30**, 1503 and references cited therein.

part of the solution of the carbanion with ^2H_2O and assaying the deuterium content of the derived substituted 1,3-dithian by ^1H n.m.r. spectroscopy. For some dithians [2-(4-nitrophenyl)-1,3-dithian (1h) in THF–hexane, (1d and a) in HMPA–THF–hexane]



(1) (2)

a; R=n-C_9H_{19}
b; R=n-C_7H_{15}
c; R=Me
d; R=Et
e; R=Bu^t
f; R=Ph
g; R=4-$MeOC_6H_4$
h; R=4-$NO_2C_6H_4$

k_H/k_D could not be measured due to destruction of the carbanion. Instability of the carbanion (2h) was surprising, as it was expected that the nitrophenyl group would provide extra stabilisation of this carbanion. On addition of butyl-lithium–hexane to a solution of (1h) in THF at 0°, there was a rapid colour change through red to black. [Species (1a—g) did not show this change.] After 7 h at room temperature the black mixture was quenched with H_2O–^2H_2O and processed in the usual way, but no (1h) or [^{2}H]-(1h) was recovered. For quenching of carbanions prepared in THF–hexane the isotope effects are all <1 with the exception of the carbanion from (1d) where it is slightly >1. The value for (1g) in HMPA–THF–hexane is significantly >1. We chose the series (1a—h) in order to examine the effects on the magnitude of the isotope effect of changing the size of the substituent at C-2 and its electronic nature. Unfortunately, no clear pattern emerges. The failure to obtain values for (1h) prevents an assessment of the electronic effect of the substituent at C-2. On the basis of these results, we do not wish to speculate on the origins of the observed isotope effects. Inverse isotope effects have been previously noted in the protonation of carbanions and possible explanations have been offered.[26] Our results have practical value with regard to the efficiency of incorporation of deuterium or tritium in quenchings of bis(thio)carbanions.[27]

After our work was completed, Caspi and Eck [28] reported a similar method to that described for the synthesis of (*S*)-[^{3}H]decan-1-ol, for the preparation of (*S*)-[^{2}H]- and (*S*)-[^{3}H]-octan-1-ol. Cornforth has recently detailed a method for establishing the stereochemistry of methyl group hydroxylation which is similar to that described in this paper.[29]

EXPERIMENTAL

Carbon tetrachloride, cyclohexane, and dioxan (stored under nitrogen) were spectroscopic grade. HMPA was purified as described.[30] Other solvents were purified just before use by standard procedures [31] (ether and THF were redistilled from $Li[AlH_4]$). The following labelled reagents were used as supplied: tritiated water (Radiochemical Centre, Amersham), deuterium oxide (from Norsk Hydro-Electrisk, nominally 99.8% ^{2}H, found by ^{1}H n.m.r. 99.65%), $Li[Al^2H_4]$ (Ciba–Geigy, >99 atom % ^{2}H). n-Butyllithium in hexane (Koch–Light or Cambrian) was standardised according to refs. 32 and 33. NAD^+, NADH, and LADH were from Boehringer or Sigma. Other reagents were commercially available compounds either of sufficient purity for direct use or were purified by standard procedures.[31]

In synthetic work, solvents were removed with a rotary evaporator (bath temperature <25°). Concentrations of solutions for ^{1}H n.m.r. spectroscopy (tetramethylsilane reference) were *ca.* 10% and for i.r. spectroscopy *ca.* 2%. U.v. spectra were recorded in cyclohexane. Electron impact mass spectra were run by PCMU, Harwell. T.l.c. plates were prepared from Machery and Nagel silica gel N. Spots were detected with iodine vapour or 35% aqueous H_2SO_4–charring.

G.l.c. analyses were carried out on a 6 ft 10% E301 column (flow rate *ca.* 40 cm^3 He min^{-1} at 150°). M.p.s were determined for samples in open capillaries and are uncorrected. Counting of tritiated samples was done with a Packard Tricarb counter (model 4322). A properly diluted sample (1 μl) was added to 18mM-2,5-diphenyloxazole (PPO) and 0.14mM-1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP) in sulphur-free toluene (10 cm^3) (*cf.* ref. 34), except in the case of tritiated water which was dissolved in toluene–Triton X-100 (2 : 1 v/v) (10 cm^3) containing 18mM-PPO and 0.14mM-POPOP. Counting efficiency was 43% for all samples and the 95/100 proportional error was <5%.

Enzyme Assays.—Concentrations of LADH in phosphate buffer (pH 7.0, μ 0.1M) were determined according to ref. 35. The assays were carried out with a Pye–Unicam SP 1800 recording u.v. spectrophotometer and with a Tachometer (Boehringer).

The change in *A* at 366 nm from 15 to 30 s was taken for calculations of initial rates for LADH.[36] Final concentrations were as follows: (a) ethanol oxidation: ethanol 9mM, NAD^+ 0.1mM, glycine–NaOH buffer pH 9.0, μ 0.1M, proper amounts (<10 μl) of LADH to give a change in *A*, Δ(*A*), of *ca.* 0.04 min^{-1}; (b) acetaldehyde reduction: acetaldehyde 1mM, NADH 0.112mM, phosphate buffer pH 7.0, μ 0.1M, proper amounts of LADH to give Δ(*A*) *ca.* 0.04 min^{-1}; (c) decanal reduction: decanal 5μM (3.2 cm^3 of a solution of 1 μl pure decanal in 1 dm^3 phosphate buffer pH 7.0, μ 0.1M), NADH 0.112mM, dilute amounts of LADH to give Δ(*A*) *ca.* 0.016 min^{-1}; (d) inhibition experiments

[26] K. B. Wiberg, *J. Amer. Chem. Soc.*, 1955, **77**, 5987; Y. Pocker and J. H. Exner, *ibid.*, 1968, **90**, 6764.
[27] An alternative, elegant method, which largely surmounts the problem of isotope effects is to quench the 2-anion of 1,3-dithians with $CF_3CO_2{}^3H$ [readily prepared from ^3H_2O and $(CF_3CO)_2O$], A. R. Battersby, personal communication.
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[35] K. Dalziel, *Biochem J.*, 1961, **80**, 440.
[36] A. D. Merit and G. T. Tomkins, *J. Biol. Chem.*, 1959, **234**, 2778.

were performed by adding different amounts of the inhibitor under study to solutions containing the substrate (ethanol or acetaldehyde) and coenzyme in concentrations as above. All assays were performed at 25° in duplicate and initiation of reactions was always done by the addition of LADH.

The specific activity of LADH with decanal at 5μM was 4.5 times lower than that of acetaldehyde (at its optimum concentration). With decanal we were working in a region where the reaction follows first-order kinetics with respect to the decanal, whereas with acetaldehyde we were working where the reaction is zero-order with respect to acetaldehyde. If the optimum concentration of decanal could be achieved, it would be a better substrate than acetaldehyde.

2-*Nonyl*-1,3-*dithian* (1a).—Dry hydrogen chloride was bubbled for 20 min into a solution of decanal (15.6 g, 0.1 mol) and propane-1,3-dithiol (11.8 g, 0.11 mol) in dichloromethane (75 cm³). A precipitate appeared which, on stirring, dissolved. The mixture was refluxed with stirring for 1 h, after which it was allowed to cool to room temperature. Washing with water (2 × 25 cm³), 10% aqueous KOH (3 × 25 cm³), and water (2 × 35 cm³) followed by filtration through anhydrous Na_2SO_4–charcoal gave a liquid which, after solvent removal and distillation under reduced pressure, afforded 2-*nonyl*-1,3-*dithian* (14.6 g, 60%), b.p. 114—116° at 0.03 mmHg, n_D^{24} 1.5600; pure by t.l.c.; δ(CCl_4) 0.94 (m, CH_3), 1.1—1.7 (m, 8 × CH_2), 1.99 (m, 4-H_2), 2.75 (m, 3- and 5-H_2), and 3.90 (t, *J* 6.7 Hz, CH), $\lambda_{max.}$(cyclohexane) 249 nm (ε 617) (Found: C, 63.4; H, 10.5; S, 26.0. $C_{13}H_{26}S_2$ requires C, 63.35; H, 10.6; S, 26.0%).

2-*Heptyl*-1,3-*dithian* (1b).—This was prepared analogously to (1a) in 53% yield, b.p. 98—100° at 0.07 mmHg, n_D^{29} 1.5060 (Found: C, 60.55; H, 10.3. $C_{11}H_{22}S_2$ requires C, 60.5; H, 10.15%).

[2-³H]-2-*Nonyl*-1,3-*dithian*.—This experiment was carried out under dry nitrogen. To a solution of 2-nonyl-1,3-dithian (3.946 g, 16 mmol) in dry THF (30 cm³), cooled to −5°, was added slowly with vigorous stirring, a solution (9.6 cm³, 13 mmol) of n-butyl-lithium in hexane. After stirring for 7 h at −5 to 0°, the mixture was treated with *ca.* 0.2 cm³ (*ca.* 11 mmol, calculated as H_2O) tritiated water (specific activity 13.3 Ci mol⁻¹), added dropwise with vigorous stirring, and then left to stir at room temperature for 2 h. Dilute hydrochloric acid (15 mmol) was added to dissolve lithium hydroxide. Removal of solvent was followed by extraction with dichloromethane–n-pentane (1 : 1 v/v; 2 × 150 cm³). The organic phase was washed with saturated aqueous sodium hydrogencarbonate (60 cm³) and water (60 cm³) and was dried ($KHCO_3$). Filtration and removal of solvents followed by column chromatography [neutral alumina, n-pentane–ether (25 : 1 v/v; first 250 ml)] gave [2-³H]-2-nonyl-1,3-dithian (3.827 g, 97%) pure by ¹H n.m.r. and t.l.c. (benzene), specific activity 35 Ci mol⁻¹.

[2-²H]-2-*Nonyl*-1,3-*dithian*.—This was prepared analogously to [2-³H]-(1a) in 92% yield, ²H content >99% by ¹H n.m.r. spectroscopy (no triplet at δ 3.90), *m/e* 249 (3.6%), 248 (5.8), 247 (25.3, M^+), 246 (1.4), and 120 (100).

[2-³H]-2-*Heptyl*-1,3-*dithian*.—This was prepared analogously to [2-³H]-(1a) in 94% yield (specific activity 37.4 Ci mol⁻¹) using tritiated water of specific activity 19.6 Ci mol⁻¹.

[2-²H]-2-*Heptyl*-1,3-*dithian*.—This was prepared analogously to [2-³H]-(1a) in 75% yield, ²H content >99% by ¹H n.m.r. spectroscopy.

2-*Methyl*-, -*Ethyl*-, *and* -*t-Butyl*-1,3-*dithian* (1c—e).—These were prepared from the corresponding aldehyde by the procedure of ref. 37. Spectroscopic data for these compounds agreed with reported properties.[38]

2-*Phenyl*-1,3-*dithian* (1f).—This was prepared according to ref. 10.

2-(4-*Methoxyphenyl*)-1,3-*dithian* (1g).—To a gently stirred, cooled solution of propane-1,3-dithiol (9.50 g, 81 mmol) and *p*-methoxybenzaldehyde (11.30 g, 81 mmol) in chloroform (60 cm³) dry hydrogen chloride was bubbled for 20 min. The mixture was stirred at room temperature for 18 h and then washed with water (2 × 50 cm³), 10% sodium hydroxide (1 × 50 cm³), and water (2 × 50 cm³). Drying (Na_2SO_4) and removal of solvent afforded a solid (17.9 g) which was recrystallised from methanol (200 cm³) and chloroform (60 cm³) with cooling at 0° overnight to give needles of (1g) (15.2 g, 83%), m.p. 115—116° (lit.,[39] 117—117.5°); δ(CCl_4) 2.05 (m, 4-H_2), 2.90 (m, 3- and 5-H_2), 3.75 (s, OCH_3), 5.02 (s, CH), 6.78 (d, *J* 8.7 Hz, 2 × ArH), and 7.34 (d, *J* 8.7 Hz, 2 × ArH).

2-(4-*Nitrophenyl*)-1,3-*dithian* (1h).—This was prepared analogously to (1g). Recrystallisation from ethyl acetate (80 cm³) with cooling to −20° for 5 h afforded the product (1h), yellowish crystals (7.24 g, 49%), m.p. 143—144° (lit.,[39] 140—142°); δ($CDCl_3$) 2.12 (m, 4-H_2), 3.05 (m, 3- and 5-H_2), 5.25 (s, CH), 7.65 (d, *J* 8.7 Hz, 2 × ArH), and 8.23 (d, *J* 8.7 Hz, 2 × ArH) (Found: C, 49.8; H, 4.6; N, 5.8. $C_{10}H_{11}NO_2S_2$ requires C, 49.8; H, 4.6; N, 5.8%).

Measurement of Kinetic Isotope Effect: General Procedure.—To a solution of the dithian (1a—h) in dry THF (*ca.* 0.35M), cooled to −5° and under dry nitrogen, a slight excess (1—3%) of n-butyl-lithium in hexane was added slowly with vigorous stirring. The ratio THF : n-hexane varied from 2 to 3.3 depending on the molarity of n-butyl-lithium used. The generation of the carbanions (2a—h) was complete usually within 7 h at −5°, after which time the solution was divided into three or four equal portions.

To one portion ^2H_2O (10-fold molar excess) was added at once with vigorous stirring, and to the other portions a preformed mixture of ^2H_2O–H_2O (0.888—0.969 molar ratio; 10-fold molar excess over the carbanion) was added at once with vigorous stirring.

The quenched systems were stirred at room temperature for 2 h and then dilute hydrochloric acid was added to dissolve lithium hydroxide. Removal of solvents was followed by extraction with dichloromethane, washing with water to neutrality (litmus), and drying (Na_2SO_4 or $KHCO_3$).

Further purification was effected either by column chromatography (alumina, neutral, grade I, Merck) or recrystallisation. Products were homogeneous by t.l.c. Deuterium incorporation in the controls was >99% by ¹H n.m.r. except in the cases of (1c) (91%) and once for (1a) (92%). Deuterium incorporation in the competitive runs was determined from the integrals of the ¹H n.m.r. spectra. This figure was corrected from the control reaction (when necessary) and the kinetic isotope effect was calculated from formula (1).[26]

[1-³H]*Decanal*.—This experiment was performed under dry nitrogen. 2-Nonyl-2-tritio-1,3-dithian (1.234 g, 5

[37] D. Seebach, *Synthesis*, 1969, 17.
[38] S. Oae, W. Tagaki, and A. Ohno, *Tetrahedron*, 1964, **20**, 427.
[39] J. H. Bowie and P. Y. White, *Org. Mass Spectrometry*, 1969, **2**, 611.

mmol) was partially dissolved in methanol–water (9 : 1 v/v; 35 cm³). Solid mercury(II) oxide (1.624 g, 7.5 mmol) was

$$k_H/k_D = \frac{\%\ ^2H \text{ in solvent}}{\%\ H \text{ in solvent}} \times \frac{\%\ H \text{ in product}}{\%\ ^2H \text{ in product}} \quad (1)$$

added, followed by a solution of mercury(II) chloride (2.986 g, 11 mmol) in the same solvent mixture (15 cm³). The reaction was refluxed with vigorous stirring for 5 h (bath temperature 80—85°) after which it was allowed to cool to room temperature. T.l.c. (silica gel, benzene) showed complete reaction of [2-³H]-(1a). Hydrolysis of the intermediate 1,1-dimethoxy-1-tritiodecane was carried out as follows: the cold reaction mixture was filtered through Celite and MeOH was removed. Sulphuric acid (25 ml, 0.01M) was added and the mixture was refluxed with vigorous stirring, under nitrogen, for 3 h (bath temperature 115—120°). After cooling to room temperature, dichloromethane–n-pentane (1 : 1 v/v; 125 cm³) was added and the mixture was washed with half-saturated aqueous ammonium acetate (2 × 50 cm³) and saturated aqueous ammonium acetate (2 × 25 cm³). The organic phase was dried (Na_2SO_4). Filtration, removal of solvents, and column chromatography [silica gel, diethyl ether–n-pentane (1 : 3 v/v)] (first 100 cm³) gave [1-³H]decanal [0.642 g, 82%, pure by ¹H n.m.r. and t.l.c. (silica gel, benzene)], specific activity 44 Ci mol⁻¹.

[1-²H]*Decanal*.—This was prepared analogously to [1-³H]-decanal, *i.e.* using ^2H_2O instead of ^3H_2O in 66% yield, b.p. 72—74° at 2.5 mmHg; no triplet at δ 9.67, $\nu_{max.}$(neat) 2 066 cm⁻¹ (C–²H).

(S)-[1-³H]*Decan*-1-*ol*.—[1-³H]Decanal (20 μl, 16.6 mg, 0.106 mmol) in dioxan (3.8 cm³, 45.6 mmol) and NADH (0.1 g, 0.115 mmol) were added to phosphate buffer (pH 7.0, 0.1M; 500 cm³) at *ca.* 25°. LADH (5.6 units; Sigma) was added and the mixture was stirred gently. The reaction was complete after 10 min. At this point more enzyme (2 units) was added and stirring was continued for a further 20 min. Unlabelled decanol (3.3897 g, pure by g.l.c.) was added as carrier. Extraction with n-pentane (5 × 100 cm³), drying (Na_2SO_4), filtration, and removal of solvent afforded [1-³H]decanol (3.4040 g, *ca.* 100% recovery), pure by ¹H n.m.r., specific activity 0.126 Ci mol⁻¹.

(S)-[1-³H]*Decyl Toluene*-p-*sulphonate*.—In a dry, stoppered flask, were added (*S*)-[1-³H]decan-1-ol (1.761 g, 11.15 mmol) and dry pyridine (3.5 cm³, 43.5 mmol), and the solution was cooled to 0°. Toluene-*p*-sulphonyl chloride (2.86 g, 15 mmol) was added in portions over 30 min and the mixture was stirred at 0° for 3 h. Water (*ca.* 1 cm³) was added dropwise over 15 min followed by pyridine (10 cm³) and ice-cooled water (60 cm³). Extraction of the aqueous layer with ether (2 × 30 cm³) and washing of the combined organic phases with ice-cooled concentrated hydrochloric acid–water (1 : 1 v/v; 2 × 20 cm³) and ice-cooled water (5 × 20 cm³) till pH 7.0 (litmus), gave a pale yellow liquid. Drying over potassium carbonate–Norite A, removal of ether, and drying *in vacuo* gave the product (3.068 g, 88%), pure by t.l.c. (alumina; benzene) and ¹H n.m.r., specific activity 0.127 Ci mol⁻¹.

Decyl Toluene-p-*sulphonate*.—This was prepared analogously to (*S*)-[1-³H]decyl toluene-*p*-sulphonate in 77—86% yield, n_D^{21} 1.4860; δ(CCl_4) 0.94 (m, CH_2CH_3), 1.22 (s, 8 × CH_2), 2.42 (s, $ArCH_3$), 3.92 (t, *J* 6 Hz, CH_2O), 7.25 (d, *J* 8 Hz, 2 × ArH), 7.68 (d, *J* 8 Hz, 2 × Ar*H*) (Found: C, 65.9; H, 8.9. $C_{17}H_{28}O_2S$ requires C, 65.3; H, 9.0%).

(*R*)-[1-²H,1-³H]*Decane*.—(1*S*)-[1-³H]decyl tosylate (3 g, 9.6 mmol) was added to a solution of Li[Al^2H_4] (0.405 g, 9.6 mmol) in dry ether (200 cm³) and the mixture was refluxed for 97 h (bath temperature 65°). Decomposition of excess of Li[Al^2H_4] was done by careful addition of 1.5M-sulphuric acid (20 cm³). Filtration through Celite gave a clear solution, which was extracted with n-pentane (4 × 30 cm³) and dried ($MgSO_4$). Removal of solvent and column chromatography [neutral alumina; light petroleum (b.p. 40—60°)] (first 100 cm³) yielded (*R*)-[1-²H,1-³H]decane (1.107 g, 81%), pure by n.m.r., specific activity 0.095 Ci mol⁻¹. Deuterium incorporation was measured by g.l.c.–m.s. at ion source 200°, electron energy 10 eV, trap current 100 μA: *M* − 2, *ca.* 0.70; *M* − 1, 0.84; *M* (*m/e* 143), 88.59; *M* + 1, 10.06; and *M* + 2, 0.60%.

1,1,10,10-*Tetradeuteriodecane*-1,10-*diol*.—Li[Al^2H_4] (1 g, 23.8 mmol) was suspended in dry ether (90 cm³). Diethyl sebacate (6.155 g, 23.8 mmol) in dry ether (50 cm³) was added dropwise over 2 h with stirring at 0 °C. After the addition, the mixture was refluxed for 1 h. 0.17M-Sulphuric acid (15 cm³) was added dropwise to the cooled mixture and left stirring until a clear ether layer resulted. The ether phase was separated and the aqueous one, after filtration (Celite), was extracted with ether (2 × 25 cm³) and all ether phases were combined. Evaporation of ether afforded crude diol which was recrystallised from benzene once to give the product (3.777 g, 89%), m.p. 72.5° [lit.,[40] (undeuteriated) 71.5°], pure by t.l.c. (alumina; chloroform), δ($CDCl_3$) 1.28 (δ, 8 × CH_2), 1.50 (s, 2 × OH), no multiplet at 3.58 (CH_2OH); $\nu_{max.}$ (hexachlorobuta-1,3-diene mull) 2 183 and 2 095 cm⁻¹ (C–²H).

1,1,10,10-*Tetradeuteriodecane*-1,10-*diyl Bistoluene*-p-*sulphonate*.—The above tetradeuteriodiol (3.740 g, 21 mmol) was dissolved in dry pyridine (40 cm³, 0.495 mol). Toluene-*p*-sulphonyl chloride (10.3 g, 54.8 mmol), m.p. 69°, was added portionwise at 0° and the mixture was stirred for 3 h at 0°. Water (*ca.* 1 cm³) was added dropwise over 15 min and then the mixture was poured into ice-cooled water (100 cm³). Filtration, washing with a little cold water, and drying by suction afforded the crude ditosylate. Recrystallisation from MeOH (*ca.* 400 cm³) gave the compound contaminated with MeOH. Drying in high vacuum at 90° for 2 days yielded the product (9.340 g, 91%), pure by t.l.c. (alumina; chloroform) and ¹H n.m.r., m.p. 102° (lit.,[41] 109—109.5°); δ($CDCl_3$) 1.19 (s, 6 × CH_2), 1.60br (2 × CH_2), 2.74 (s, 2 × $ArCH_3$), 7.33 (d, *J* 8 Hz, 2 × ArH), 7.78 (d, *J* 8 Hz, 2 × ArH), no triplet at 3.91 (CH_2OTos); $\nu_{max.}$ (hexachlorobuta-1,3-diene mull) 2 252 cm⁻¹ (C–²H).

1,1,10,10-*Tetradeuteriodecane*.—The above ditosylate (9.26 g, 19 mmol) was added to a suspension of Li[AlH_4] (0.76 g, 20 mmol) in dry ether (400 cm³), at room temperature and the mixture was refluxed with stirring for 200 h. After cooling to room temperature, 3.1M-sulphuric acid (25 cm³) was added dropwise and the mixture was left stirring until a clear ether phase resulted. The ether phase was separated and the aqueous layer, after filtration through Celite, was extracted with n-pentane (2 × 30 cm³). The organic phases were combined and solvents were removed. The crude product was chromatographed [neutral alumina; light petroleum (b.p. 40—60°)] (first 200 cm³) to yield the product (2.13 g, 77%), pure by g.l.c., ¹H n.m.r., and i.r.;

[40] F. W. Schueler and C. Hanna, *J. Amer. Chem. Soc.*, 1952, **74**, 2112.

[41] E. J. P. Fear, J. Thrower, and J. Veitch, *J. Chem. Soc.*, 1958, 1322.

$\delta(CCl_4)$ 0.99br (2 × CD_2H) and 1.24 (s, 8 × CH_2), $\nu_{max.}$(neat) 2 931, 2 894, 2 216, and 2 126 (C–^{2}H stretching), 1 461, and 1 290 cm^{-1}. Peaks at 2 960 (asymmetric CH_3 stretching) and 2 874 (symmetric CH_3 stretching) were absent and the 1 379 (symmetric deformation of CH_3) was moved to 1 290 cm^{-1}. Deuterium incorporation was measured under the same conditions as in (*R*)-[1-^{2}H,1-^{3}H]decane: $M - 4$, 0.39; $M - 3$, 0.11; $M - 2$, 1.21; $M - 1$, 2.84; M (*m/e* 146), 85.96; $M + 1$, 10.05; $M + 2$, 0.51%.

We thank the S.R.C. for financial support and acknowledge useful discussion with Sir John Cornforth.

[7/1553 *Received*, 31st *August*, 1977]