Studies of Enzyme-mediated Reactions. Part 10.[1] Stereochemical Course of the Dehydrogenation of Stereospecifically Labelled 1-Aminoheptanes by the Amine Oxidase from Rat Liver Mitochondria (E.C. 1.4.3.4)

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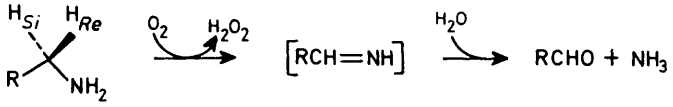
(1*R*)- and (1*S*)-[1-$^3H_1$]aminoheptanes are synthesised by a route which, in the deuterium series, allows direct correlation with the primary standard, monodeuteriosuccinic acid. The amines, thus of proven absolute stereochemistry, are then used as substrates for the monoamine oxidase from rat liver mitrochondria. The results prove that the (*Re*)-hydrogen (Scheme 1) is stereospecifically removed by the enzyme. Some anomalous values for retention of tritium found in these experiments lead to the studies described in the following paper.

AMINE oxidases [2,3] form a widely distributed family of enzymes which utilise molecular oxygen to bring about the conversion of a primary amine into an aldehyde, probably as in Scheme 1; see ref. 3 for evidence in



SCHEME 1

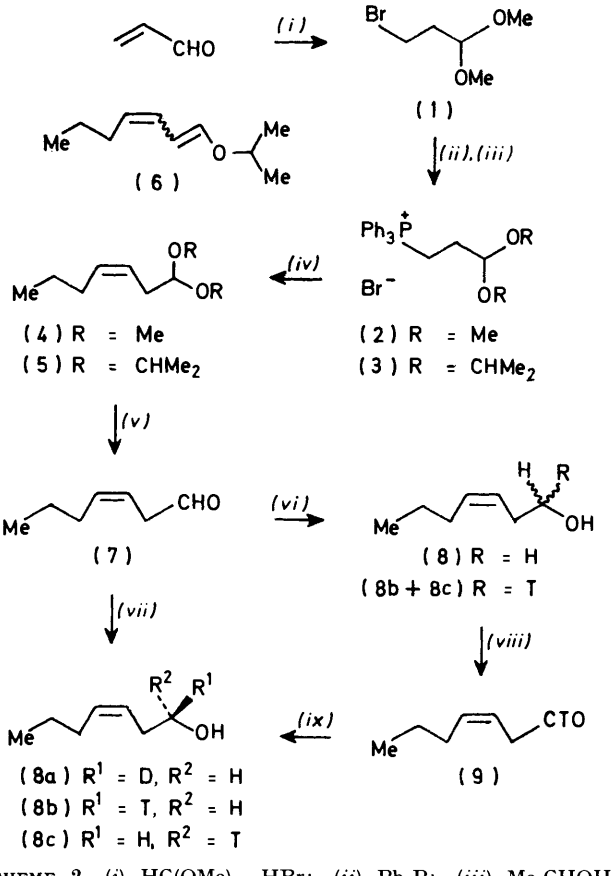
support of the illustrated imine as an intermediate. The precise mechanism almost certainly varies since some amine oxidases require Cu^{II} whereas others use the flavin coenzyme FAD.[2,3] The diamine oxidase from pea seedlings in the former group was shown [4] to remove the *Si*-hydrogen † from the methylene of benzylamine. We wished to compare this result with the stereospecificity of an amine oxidase in the second (FAD) group and chose the monoamine oxidase from rat liver mitochondria [5] (E.C. 1.4.3.4.). This enzyme was also used in the pioneering studies by Belleau and his co-workers [6] on its dehydrogenation of tyramine; the correlation of our work with theirs will be covered in Part 12 of this series.

1-Aminoheptane was chosen as the substrate for the present work because it appeared [2,3,5] to be suitable for both the mitochondrial enzyme and for future studies on the oxidase from beef plasma.[7]

Syntheses of Labelled Substrates.—The route to the various 1-aminoheptanes, made chiral by isotopic substitution, is shown in Schemes 2 and 3. A key intermediate was the β,γ-unsaturated aldehyde (7); it will be seen later that the double bond at C-3,4 was an important feature to allow unambiguous determination of the absolute stereochemistry at C-1 of the final labelled 1-aminoheptanes.

The synthesis of (*Z*)-hept-3-enal (7) is set out in Scheme 2. Treatment of acrolein with methanolic hydrogen bromide and an equimolar amount of trimethyl orthoformate [8] gave the bromoacetal (1) in 58% yield. This reacted with triphenylphosphine and the product (2) was converted by isopropyl alcohol and sulphuric acid into the isopropyl acetal (3). Then a Wittig reaction [9] of (3) with butanal gave a mixture of olefins, from which the major product (5) could be isolated by preparative g.l.c. in *ca.* 25% overall yield. A minor fraction was shown by n.m.r. to contain the enol ether(s) (6).

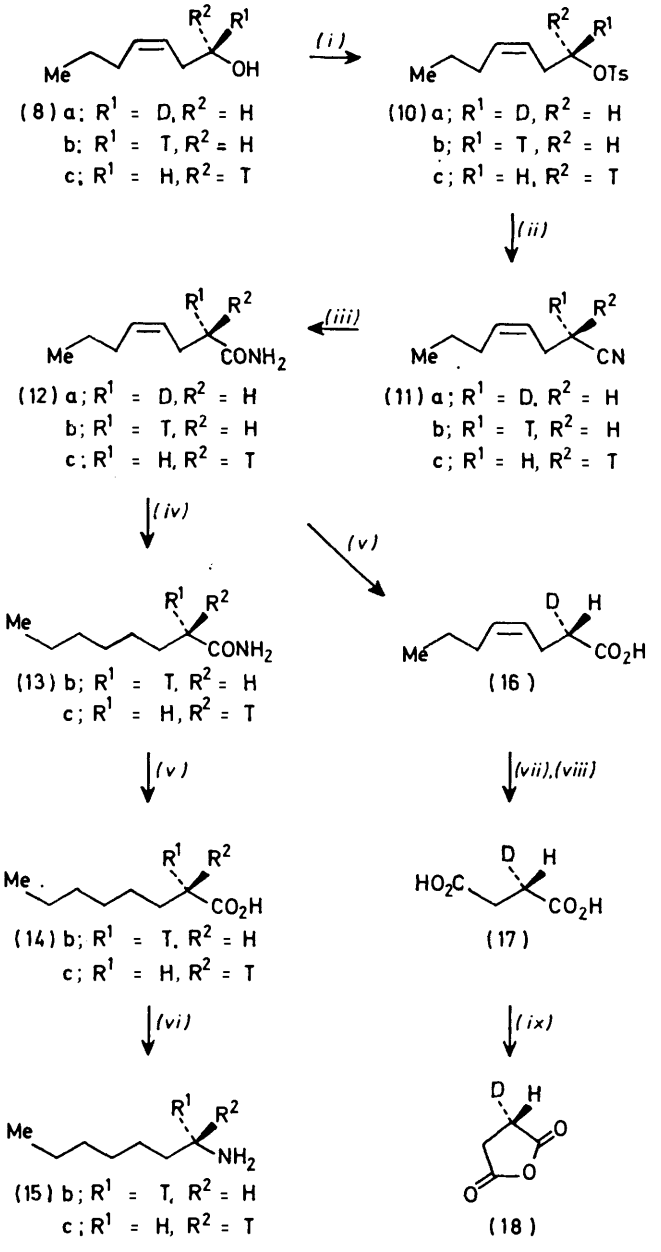
Initially, the dimethyl acetal (2) had been converted directly into the acetal (4) but we were unable to hydrolyse the acetal function without isomerising some of the



SCHEME 2 (*i*) $HC(OMe)_3$, HBr; (*ii*) Ph_3P; (*iii*) Me_2CHOH, H_2SO_4; (*iv*) $MeSOCH_2^-Na^+$–Me_2SO, $Me[CH_2]_3CHO$; (*v*) oxalic acid–H_2O–acetone; (*vi*) $LiAlH_4$, Et_2O or $NaBH_3T$, H_2O; (*vii*) liver alcohol dehydrogenase, NAD^+, and $MeCD_2OH$ or MeCHTOH; (*viii*) CrO_3–pyridine complex, CH_2Cl_2; (*ix*) liver alcohol dehydrogenase, NAD^+, $MeCH_2OH$

† Stereochemical conventions are reviewed by J. B. Jones, in 'Techniques of Organic Chemistry,' eds. J. B. Jones, C. J. Sih, and D. Perlman, Wiley, New York, vol. X, Part I, 1976, p. 479.

material to the unwanted conjugated isomer. However, the isopropyl acetal (5) underwent rapid acidic hydrolysis, and the β,γ-unsaturated aldehyde (7) was isolated without significant isomerisation.



SCHEME 3 (*i*) TsCl, pyridine, −5 °C; (*ii*) NaCN, DMF; (*iii*) H_2O_2, NaOH, EtOH, 50 °C; (*iv*) H_2–Pt, $MeCO_2Et$; (*v*) 0.5N-H_2SO_4, 100 °C; (*vi*) H_2SO_4, $CHCl_3$, NaN_3; (*vii*) O_3, −78 °C; (*viii*) H_2O_2, HCO_2H, 100 °C; (*ix*) MeCOCl

Immediate reduction of the aldehyde (7) with lithium aluminium hydride gave (*Z*)-hept-3-en-1-ol (8) which was characterised as its known [10] biphenyl-4-ylurethane derivative. The Experimental section gives an improved procedure for the preparation of the necessary reagent, biphenyl-4-ylisocyanate.[11] Direct comparison of our product with authentic samples of the biphenyl-4-ylurethane derivatives of (*Z*)- and (*E*)-hept-3-en-1-ol,[10] kindly supplied by Professor L. Crombie, confirmed the indications from n.m.r. spectra that the present product had the (*Z*)-stereochemistry (8); the corresponding aldehyde thus has structure (7).

Formation of the (*Z*)-olefin as the major product in such a Wittig reaction is interesting; there are analogies, however, for cases involving non-stabilised ylides reacting with non-conjugated aldehydes especially in dimethyl sulphoxide solution.[12]

The syntheses of the required labelled 1-aminoheptanes (Schemes 2 and 3) were developed using deuterium labelled compounds. Freshly prepared (*Z*)-hept-3-enal (7) was reduced to the (1*R*)-alcohol (8a) by stereospecific transfer of deuterium from [1,1-$^2H_2$]ethanol in a reaction mediated by liver alcohol dehydrogenase in the presence of a catalytic quantity of the coenzyme NAD^+.[13] Treatment of the alcohol (8a) with toluene-4-sulphonyl chloride in pyridine at −5 °C gave the toluene-4-sulphonyl derivative (10a) which was converted immediately into the nitrile (11a).

Direct hydrolysis of the nitrile to the corresponding carboxylic acid was not followed because of the risk of racemisation. The alternative base-catalysed reaction of a nitrile with hydrogen peroxide [14] was therefore used but under the usual conditions gave low yields of the desired amide (12a), together with a mixture of other, more polar products. These may have arisen by interaction of the olefin group with the intermediate peroxycarboximidic acid.[14] Accordingly, the reaction was run in the presence of a very reactive olefin (α-pinene) when the required amide (12a) was obtained in 63% yield.

If the above introduction of deuterium [(7)→(8a)] occurs on the *Re*-face of the aldehyde [13] and if the displacement step [(10a)→(11a)] takes place with inversion, then the product will have the (1*S*)-configuration (12a). However, it was imperative to have an independent proof of the absolute configuration of the amide, as well as a measure of its stereochemical purity. It was for this reason that the 3,4-double bond had been built into the molecule initially, since it allowed ready degradation to monodeuteriosuccinic acid (17), one of the primary standards in this area.[15] Direct ozonolysis of the unsaturated amide (12a) gave a mixture of products in poor yield, so prior hydrolysis to the corresponding carboxylic acid (16) was effected with hot aqueous sulphuric acid. Ozonolysis of the acid (16) gave (+)-[2-$^2H_1$]succinic acid. Importantly, the o.r.d. measurements, kindly carried out by Professor Sir John Cornforth and Dr. G. Ryback (Table 3, Experimental section), showed that the deuteriosuccinic acid contained 94 ± 5% of the (2*S*)-isomer (16). Mass-spectrometric examination [16] of the derived anhydride (18) showed it to contain ⩾0.95 ± 0.03 deuterium atoms per molecule.

With the synthetic base thus firmly established, the procedures were then adapted to produce the various tritiated 1-aminoheptanes required for the enzymic

experiments. First, (1*RS*)-[1-$^3H_1$]ethanol * (prepared by reduction of acetaldehyde with borotritide) was used for enzymic reduction of (*Z*)-hept-3-enal (7) as above to yield (1*R*)-(*Z*)-[1-$^3H_1$]hept-3-en-1-ol (8b). Subsequent steps (8b)→(10b)→(11b)→(12b) paralleled exactly the steps described above for the deuteriated series.

The starting material for the enantiomer (12c) was (*Z*)-[$^3H_1$-formyl]hept-3-enal (9), which was prepared from unlabelled material (7) by reduction with sodium borotritide followed by re-oxidation with chromium trioxide–pyridine;[18] the ^{3}H-isotope effect operated here to our advantage. This aldehyde (9) was then reduced by liver alcohol dehydrogenase and unlabelled ethanol to the (1*S*)-alcohol (8c). The chemically equivalent sequence (8c)→(10c)→(11c)→(12c) was then followed as before.

Hydrogenation of the amides (12b) and (12c) to the saturated amides (13b) and (13c), followed by mild acidic hydrolysis, then afforded (1*S*)-[2-$^3H_1$]octanoic (14b) and (1*R*)-[2-$^3H_1$]octanoic acid (14c). Finally, these were converted by Schmidt degradation into (1*S*)-1-amino-[1-$^3H_1$]heptane (15b) and (1*R*)-1-amino[1-$^3H_1$]heptane (15c), respectively.

It should be emphasised that the illustrated absolute configurations for amines (15b and c) are secure and both materials are of high configurational purity. This follows from the work in the deuterium series coupled with the knowledge that the Schmidt rearrangement always occurs with retention at the migrating centre.[19]

Finally, the racemic compound (1*RS*)-1-amino[1-$^3H_1$]-heptane (15b and c), was prepared by reduction of the aldehyde (7) with sodium borotritide to produce (1*RS*)-(*Z*)-[1-$^3H_1$]hept-3-en-1-ol (8b and c), which was converted into the labelled amine by the now standard route.

The use of parallel equivalent syntheses to prepare the required labelled amines is particularly important because it ensures that the two enantiomers not only have opposite configurations but also have the same degree of configurational purity; they should thus produce strictly complementary results in the enzymic experiments.

Enzymic Studies.—Monoamine oxidase (E.C. 1.4.3.4.) has been found in all classes of vertebrates so far examined.[2,3] The enzyme occurs in many different tissues, particularly in glands, plain muscle, and the nervous sytem;[20] a high activity of monoamine oxidase is always found in the liver.[2,3,21] The monoamine oxidase from rat liver mitochondria has been studied extensively,[3] especially by Tipton [2] and Sourkes.[22,23] The enzyme does not require Cu^{II} or pyridoxal phosphate but FAD has been shown to be an essential co-factor. Mitochondrial monoamine oxidases are difficult to purify, and substantial losses in activity usually occur during purification. However, partially purified preparations [2,3] of the rat liver enzyme † were suitable for the present stereochemical investigations. The activity of each preparation (measured spectrometrically with benzylamine as substrate by following the formation of benzaldehyde) was found to be between 0.1 and 1 μmol benzylamine oxidised per min per mg of protein at 30° C.

For the enzymic experiments, it was necessary to consider the susceptibility of the product heptanal to further oxidation, particularly since oxygen is required by the amine oxidase and hydrogen peroxide is a by-product (Scheme 1). If partial oxidation of the aldehyde occurred, the ^{3}H-specific activity of the residual heptanal would be increased as a result of the kinetic isotope effect. The aldehyde was therefore reduced *in situ* to heptan-1-ol by a coupled redox reaction based on liver alcohol dehydrogenase with ethanol as the hydrogen donor; the enzyme catalase was added to remove hydrogen peroxide as it was formed.

Each of the three tritiated amines (15b), (15c), and (15b and c) was incubated separately with the mixed enzyme system, and after 20 h the product heptanol was isolated. Experiments 3 and 4 (Table 1) strongly

TABLE 1

Incubation of tritiated 1-aminoheptanes with the amine oxidase

			Heptan-1-ol [b]	
Expt. no.	1-Aminoheptane [a]	Specific activity [c]	Specific activity [c]	% of original specific activity
1	(1*S*)-[1-$^3H_1$]-(15b)	1.04	1.67	160 ± 5
2		0.939	1.48	158 ± 5
3	(1*R*)-[1-$^3H_1$]-(15c)	0.906	2.4×10^{-2}	2.5 ± 1
4		0.936	1.75×10^{-2}	1.9 ± 0.5
5	(1*RS*)-[1-$^3H_1$]-(15b and c)	3.25	1.73	53 ± 3

[a] Radioassay as *N*-heptylurea. [b] Radioassay as the biphenyl-4-ylurethane. [c] μCi mmol^{-1}.

indicated that the (*Re*)-hydrogen atom had been removed stereospecifically in the oxidative deamination reaction, but the unexpectedly high values for tritium retention for the (1*S*)-amine were puzzling ‡ (Experiments 1 and 2, Table 1). The following paper [25] describes results which allowed the anomaly to be understood; this work also indicated the way to minimise this increase in tritium level and these conditions were used for the studies which follow using doubly-labelled amines.

1-Amino[1-^{14}C]heptane [as (15)] was prepared as in Scheme 4, and was mixed in suitable quantity with each of the tritiated amines (15b), (15c), and (15b and c) to provide an internal standard. The doubly-labelled

* The more efficient alternative methods, using either [1-^{3}H]-cyclohexanol [17] or [1-^{3}H]cyclopentanol [4] as reducing agent, were not suitable in this case because separation of a small amount of the desired alcohol (8) from cyclohexanol or cyclopentanol was impractical.

† These preparations contain the normal mixture of amine oxidase isoenzymes, but significantly they have been shown to be free of alcohol dehydrogenase and aldehyde dehydrogenase activity.[24]

‡ The retention values were somewhat variable and the value from experiment 5, Table 1 is low relative to experiments 1 and 2. However, the main point is that unexpectedly high values were generally observed.

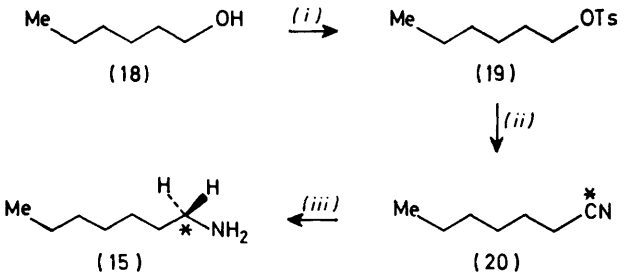
TABLE 2

Incubation of doubly labelled 1-aminoheptanes with the amine oxidase

Expt. no.	1-Aminoheptane [a]	$^3H/^{14}C$ value	Heptan-1-ol [b] $^3H/^{14}C$ value	Heptan-1-ol [b] % of original value
1	(1*S*)-[1-^{14}C,1-$^3H_1$]-(15b)	10.6 ± 0.3	12.0 ± 0.3	113 ± 6
2		10.3 ± 0.3	11.6 ± 0.3	112 ± 6
3	(1*R*)-[1-^{14}C,1-$^3H_1$]-(15c)	6.5 ± 0.2	0.59 ± 0.05	9 ± 2
4		6.7 ± 0.2	0.61 ± 0.05	9 ± 2
5	(1*RS*)-[1-^{14}C,1-$^3H_1$]-(15b + c)	21.2 ± 0.3	10.7 ± 0.3	50 ± 3
6		20.9 ± 0.3	10.5 ± 0.3	50 ± 3

[a] Radioassay as *N*-heptylurea. [b] Radioassay as the biphenyl-4-ylurethane.

amines were converted into picrates for purification and storage, and the free amine was recovered immediately prior to incubation by chromatography of the picrate on alumina. The modifications used in the enzymic runs included the omission of catalase, increase of the buffer



SCHEME 4 (*i*) TsCl–C_5H_5N, −5 °C; (*ii*) $Na^{14}CN$–DMF; (*iii*) H_2, 10%Pd–C, EtOH–HCl.

concentration, and addition of the amine substrate in small portions, freshly recovered each time, throughout the period of the reaction.

The results of these experiments are collected in Table 2. When taken with those given earlier (Table 1), they show unequivocally that the *Re*-hydrogen atom (Scheme 1) is removed stereospecifically from the methylene group during oxidative deamination by monoamine oxidase from rat liver mitochondria. The modified conditions suppressed the increase in tritium specific activity significantly, and also resulted in improvement in the recovery of heptan-1-ol. The higher values for tritium retention from the (1*R*)-amine (Experiments 3 and 4, Table 2) are probably due to slightly greater loss of optical purity in the preparation of this batch of (1*R*)-1-amino[1-$^3H_1$]heptane compared with the earlier one used for Table 1; the (*S*)- and (*RS*)-labelled amines for Table 2 were from the same samples used previously.

The *Re*-stereospecificity found here for the rat liver enzyme acting on heptylamine ties in with the work of Belleau *et al.*[6] for the action of the enzyme on tyramine. It has already proved valuable [26] to have this stereochemical probe available which works in the opposite sense to the pea-seedling enzyme [4] (*Si*-specific) and we believe this will be generally helpful for similar researches on primary amines, *e.g.* from decarboxylation of α-amino acids. Examples of our use of these two enzymes will be described in future papers.

EXPERIMENTAL

Most general directions are given in ref. 4. All commercial solvents were purified and distilled before use. Ligroin refers to that petroleum fraction which had b.p. 105—115 °C, light petroleum had b.p. 57—67 °C, and pentane had b.p. 35—37 °C. Gas–liquid chromatography (g.l.c.) was carried out on either Perkin-Elmer F21 (preparative) or F11 (analytical) instruments, using the following columns: I, a 6 m × 9-mm internal diameter copper column packed with 30% w/w Apiezon L on Chromosorb P (60—80 mesh); II, a 7.6 m × 1-mm internal diameter glass column packed with 5% w/w Carbowax on Chromosorb W (70—80 mesh).

3-Bromo-1,1-dimethoxypropane (1).—Anhydrous hydrogen bromide was passed into a tared flask containing dichloromethane (1 l) at 0 °C) until the solution was saturated (66.0 g, 0.815 mol HBr absorbed). Acrolein (45.6 g, 0.815 mol) was added in one portion to the stirred solution followed after 2 min by trimethyl orthoformate (173 g, 1.63 mol) in methanol (200 ml). Stirring was continued at 0 °C for 10 min when anhydrous calcium carbonate (20 g) was added. After 1 h, the mixture was filtered, the filtrate concentrated to *ca.* 100 ml and the residue distilled under reduced pressure to give the pure dimethyl acetal (85.5 g, 58%), b.p. 60—62 °C at 15 mmHg (lit.,[27] 58—60 °C at 17 mmHg); $\nu_{max.}$ 1 120 and 1 010 cm^{-1}; δ 4.58 (1 H, t, *J* 6.5 Hz, 1-H), 3.44 (2 H, t, *J* 6.5 Hz, 3-H_2), 3.37 (6 H, s, 2 × OMe), and 2.14 (2 H, dt, *J* 6.5 Hz, 2-H_2).

(3,3-Di-isopropoxypropyl)triphenylphosphonium Bromide (3).—Triphenylphosphine (122 g, 0.465 mol) was added to 3-bromo-1,1-dimethoxypropane (85 g, 0.465 mol) in anhydrous acetonitrile (200 ml) and the solution was heated under reflux for 4 h. The residue from evaporation of the acetonitrile *in vacuo* was treated with sulphuric acid (2 ml) in anhydrous isopropyl alcohol (1 l), and the solution was boiled gently for 26 h while a mixture of methanol and isopropyl alcohol (210 ml) distilled out through a lagged 30-cm Vigreux column under a reflux ratio of *ca.* 10 : 1, b.p. 70—82 °C. The solution was cooled, neutralised with 1% sodium isopropoxide in isopropyl alcohol, and evaporated under reduced pressure to give a gum which crystallised from benzene to give the crude phosphonium salt (184 g, 79%), m.p. 104—105.5 °C, sufficiently pure for use in the Wittig reaction; δ 7.74 (15 H, m, Ar-H), 5.12 (1 H, br t, *J* 5.5 Hz, 3-H), 3.9 (4 H, m, 1-H_2 and 2 × Me_2CH), 1.9 (2 H, m, 2-H_2), and 1.17 (12 H, d, *J* 6.5 Hz, 2 × Me_2CH).

(Z)-1,1-Di-isopropoxyhept-3-ene (5).—A solution of sodium methylsulphylide in dimethyl sulphoxide was prepared [9] under nitrogen from 50% sodium hydride dispersion in oil (14.4 g, 0.30 mol NaH) and anhydrous dimethyl sulphoxide (200 ml). (3,3-Di-isopropoxypropyl)triphenylphosphonium bromide (150 g, 0.30 mol) in dimethyl sulphoxide (350 ml) was added and the resulting red solution was stirred for 10 min at 20 °C. Butanal (21.6 g, 0.30 mol) was then added slowly, the solution was stirred for a further 4 h at 20 °C and poured into a cold mixture of water (1 l) and saturated aqueous sodium chloride (1 l). The mixture was kept at 0 °C overnight and then filtered to remove triphenylphosphine oxide. The filtrate was extracted with light petroleum–ether (1 : 1) (5 × 100 ml) and the organic

extract was washed with water, dried, and concentrated under reduced pressure to *ca.* 60 ml. Vacuum distillation gave a major fraction (34.5 g, 54%), b.p. 115—120 °C at 15 mmHg, which was mainly the required acetal by n.m.r. Preparative g.l.c. (column I, flow rate 200 ml min^{-1}, 170 °C) gave pure *acetal* (17.6 g, 25%), one peak of retention time 20.0 min in an analytical run on the same column (Found: C, 72.7; H, 11.9. $C_{13}H_{26}O_2$ requires C, 72.9; H, 12.1%); $\nu_{max.}$ 3 010, 1 650, 1 120, and 1 030 cm^{-1}; δ 5.4 (2 H, m, $W_{\frac{1}{2}}$ 9 Hz, *cis* CH=CH), 4.51 (1 H, t, *J* 6 Hz, 1-H), 3.84 (2 H, septet, *J* 6 Hz, 2 × Me_2CH), 2.31 (2 H, br t, 2-H_2), 2.0 (2 H, m, 5-H_2), 1.3 (2 H, m, 6-H_2), 1.14 and 1.09 (12 H, overlapping d + d, *J* 6 Hz, 2 × Me_2CH), and 0.86 (3 H, t, *J* 6.5 Hz, $MeCH_2$).

(Z)-*Hept*-3-*enal* (7).—The foregoing acetal (1.2 g, 5.6 mmol) was added to a solution containing water (25 ml) and 4% oxalic acid in acetone (100 ml), and the solution was allowed to stand at 20 °C for 3.5 h. The clear solution was diluted with half-saturated sodium chloride (250 ml) and extracted with pentane (1 × 100, 4 × 20 ml). The organic extract was washed with water, saturated sodium bicarbonate, and saturated brine, dried, and evaporated at 20 °C to give the aldehyde as an oil (0.65 g) which was reduced immediately as below; $\lambda_{max.}$ only end absorption; $\nu_{max.}$ 3 010, 2 730, and 1 730 cm^{-1}; δ 9.74 (1 H, t, *J* 2 Hz, 1-H), 5.65 (2 H, m, $W_{\frac{1}{2}}$ 10 Hz, *cis*-CH=CH), 3.2 (2 H, m, 2-H_2), 2.0 (2 H, m, 5-H_2), 1.3 (2 H, m, 6-H_2), 0.90 (3 H, t, *J* 6.5 Hz, $MeCH_2$).

(Z)-*Hept*-3-*en*-1-*ol* (8).—The above aldehyde (0.62 g, 5.5 mmol) in ether (25 ml) was added during 5 min to a stirred suspension of lithium aluminium hydride (0.15 g, 4.0 mmol) in ether (25 ml) and the mixture was heated under reflux for 2 h and then cooled. The unreacted hydride was removed by the addition of methyl formate (0.2 ml), followed by water (0.1 ml), 3N-sodium hydroxide (0.2 ml), and water (0.5 ml). The mixture was filtered and the solids were boiled with ether, and after filtration the residue was washed with more ether. The residue from evaporation (20 °C) of the combined filtrates and washings was distilled bulb-to-bulb (80—100 °C, 20 mm) to give (*Z*)-hept-3-en-1-ol (0.48 g, 72%); $\nu_{max.}$ (film): 3 350, 3 010, 1 655, and 1 050 cm^{-1}; δ 5.45 (2 H, m, $W_{\frac{1}{2}}$ 10 Hz, *cis*-CH=CH), 3.60 (2 H, t, *J* 6.5 Hz, 1-H_2), 2.30 (2 H, dt, *J* 6.5 Hz, 2-H_2), 2.0 (3 H, m, 5-H_2 and OH), 1.3 (2 H, m, 6-H_2), and 0.86 (3 H, t, *J* 6.5 Hz, $MeCH_2$); *m/e* 114 (M^+ 8%), 96 (15), 81 (60), 55 (100), and 41 (90), [113/114 <3%].

The alcohol (0.10 g) in ether (1 ml) was added to biphenyl-4-ylisocyanate (0.2 g) and the mixture was heated at *ca.* 100 °C for 0.5 h. The pale yellow solid (0.22 g), extracted from the product into boiling ligroin (2 ml), was purified by p.l.c. on silica [ethyl acetate–benzene (1 : 4), R_F 0.4] and recrystallised from ligroin to give the urethane as white needles (0.15 g), m.p. 84—84.5 °C (lit.,[10] 84 °C), undepressed on admixture with authentic *cis* material [10] but lowered to 70—85 °C by admixture with the *trans* isomer [10] (Found: C, 77.4; H, 7.2; N, 4.5. $C_{20}H_{23}NO_2$ requires C, 77.7; H, 7.45; N, 4.5%); $\nu_{max.}$ (KBr disc) 3 320, 1 705, and 1 240 cm^{-1}; δ 7.7—7.2 (9 H, m, Ar-H), 6.7 (1 H, br s, NH), 5.45 (2H, m, $W_{\frac{1}{2}}$ 10 Hz, *cis*-CH=CH), 4.17 (2 H, t, *J* 6.5 Hz), 2.4 (2 H, dt, 2-H_2), 2.0 (2 H, dt, 5-H_2), 1.35 (2 H, m, 6-H_2), and 0.89 (3 H, t, *J* 6.5 Hz, $MeCH_2$).

Biphenyl-4-*yl Isocyanate*.—4-Nitrobiphenyl (19.9 g, 0·10 mol) in ethanol (500 ml) was hydrogenated at 1 atm and 20 °C over Adams catalyst (0.1 g) to give 4-aminobiphenyl (10.9 g, 65%), after filtration, evaporation, and crystallisation from ether–pentane, m.p. 49—50 °C (lit.,[27] 50—52 °C). Phosgene was passed slowly into ether (1 l) at 0 °C until 300 g had been absorbed. A cold suspension of 4-aminobiphenyl hydrochloride in ether [prepared from the amine 8.45 g, 50 mmol) in ether (200 ml) and anhydrous HCl] was added over 20 min to the vigorously stirred 30% phosgene solution (*ca.* 1.2 l) and stirring was continued for a further 5 h. The bath temperature was raised to 20 °C and nitrogen was passed through the solution for 1 h while the effluent gases were passed through 5% KOH (5 l), after which the solution was concentrated by slow distillation into 5% KOH (2 l) (bath temperature 50 °C). The concentrated reaction mixture was evaporated *in vacuo* and the residue crystallised from ligroin to give biphenyl-4-ylisocyanate (5.36 g, 55%), m.p. 56—57 °C (lit.,[11a] 57 °C); $\nu_{max.}$ (Nujol) 2 270 and 2 230 (sh) cm^{-1}.

[1,1-$^2H_2$]*Ethanol*.—Freshly distilled acetyl chloride (8.15 g, 102 mmol) in dry ether (15 ml) was added slowly to a stirred suspension of lithium aluminium deuteride (2.0 g, 47.4 mmol; D_4 >99.9%) in dry ether (35 ml) at 0 °C. The mixture was heated under reflux for 4 h, cooled, and the residue from evaporation was treated with 2N-sodium hydroxide (10 ml), and distilled water (40 ml) was added gradually with swirling until the mixture had become a smooth paste. The volatile material was vacuum transferred (0.04 mmHg, bath temperature 20 °C) to give a clear distillate, shown to contain *ca.* 3.9 g of CH_3CD_2OH by n.m.r. analysis. This solution was fractionated through a Vigreux column and the distillate was collected up to 100 °C to give the dideuteriated alcohol (*ca.* 3.9 g, 89%) in water (2.5 g). Distilled water (1.4 g) was added to give *ca.* 50% w/w aqueous [1,1-$^2H_2$]ethanol (7.8 g).

(1R)-(Z)-[1-$^2H_1$]*Hept*-3-*en*-1-*ol* (8a).—Albumin (10 mg; Sigma) and NAD^+ (10 mg; free acid, Boehringer) were dissolved in a solution containing 0.2M-phosphate (K^+) buffer, pH 7.0 (25 ml), distilled water (185 ml), and [1,1-$^2H_2$]ethanol (3.5 g) in water (3.5 g). This solution was added to (*Z*)-hept-3-enal (0.36 g, 3.2 mmol) in dioxan (2 ml). The cloudy mixture was clarified by warming to 30 °C and ethanol-free horse-liver alcohol dehydrogenase (5 mg; *ca.* 1.4 units mg^{-1}; Sigma) was added. The solution was sealed under nitrogen and left for 18 h at 34 °C in the dark. Further alcohol dehydrogenase was added and the reaction mixture left for 10 h as before. The cloudy solution was saturated with sodium chloride, water (30 ml) was added and the mixture was extracted continuously with ether for 18 h. The ether extract was concentrated to *ca.* 300 ml and washed with 1% sodium hydrogen carbonate in saturated sodium chloride (2 × 10 ml), dried, and concentrated under reduced pressure (bath temperature 30 °C) to give a yellow oil (0.27 g). Bulb-to-bulb distillation at 85—95 °C and 20 mmHg gave the *monodeuteriated alcohol* (0.18 g, 50%), homogeneous by g.l.c. (column II, 110 °C, retention time 10.1 min) (Found: M^+, 115.110 4. $C_7{}^1H_{13}{}^2H_1O$ requires *M*, 115.110 7); $\nu_{max.}$ (film): 3 340, 3 010, 2 140, and 1 060 cm^{-1}; δ (CCl_4) 5.40 (2 H, m, *cis*-CH=CH), 3.50 (1 H, br t, *J* 6.5 Hz and *J* $^1H^2H$ 1.5 Hz, 1-H), 2.9 (1 H, br s, OH), 2.24 (2 H, dd, *J* 6.5 and 6.5 Hz, 2-H_2), 2.03 (2 H, dt, *J* 6.5 Hz, 5-H_2), 1.35 (2 H, m, 6-H_2), and 0.91 (3 H, t, *J* 6.5 Hz, $MeCH_2$); *m/e* 115 (M^+, 8%), 97 (17), 82 (50), 81 (30), 55 (100), and 41 (90) [114/115 <4%].

(2S)-(Z)-[2-$^2H_1$]*Oct*-4-*enonitrile* (11a).—Toluene-4-sulphonyl chloride (330 mg, 1.73 mmol) in cold pyridine (3 ml) was added to a solution of the above (1*R*)-deuteriated alcohol (8a) (160 mg, 1.4 mmol) in pyridine (2 ml) at −5 °C, and the solution was allowed to stand at 0 °C overnight. The mix-

ture was diluted with ice–water (100 g), the pH adjusted to 1 with 2N-sulphuric acid, and the mixture was extracted with pentane (1 × 50, 5 × 20 ml). The combined pentane extracts were washed with cold 1N-sulphuric acid, water, saturated sodium hydrogen carbonate, and saturated sodium chloride, dried, and evaporated *in vacuo* (bath temperature 30 °C) to give the tosylate (10a) as a colourless oil (340 mg, 91%), homogeneous on t.l.c. (SiO_2,C_6H_6, R_F 0.5); $\nu_{max.}$ (film): 2 140, 1 600, 1 175, 960, and 915 cm^{-1}; δ (CCl_4) 7.73 and 7.27 (4 H, dd, *J* 8.5 Hz, AA'BB' pattern, aryl-H), 5.35 (2 H, m, *cis*-CH=CH), 3.93 (1 H, br t, 1-H), 2.45 (3 H, s, aryl-Me), 2.4 (2 H, m, 2-H_2), 1.9 (2 H, dt, *J* 6.5 Hz, 5-H_2), 1.3 (2 H, m, 6-H_2), and 0.89 (3 H, t, *J* 6.5 Hz, *Me*CH_2).

Ice-cold, saturated sodium cyanide (hydroxide-free) in dry dimethylformamide (40 ml) was added to the above tosylate (290 mg, 1.08 mmol). Solid sodium cyanide (1.0 g) was added to the stirred solution, which was allowed to warm to 20 °C, and stirring was continued for a further 16 h. After the mixture had been diluted with ice–water (100 g), it was extracted with pentane (5 × 25 ml) and the extracts were washed with water (2 × 25 ml), saturated sodium hydrogen carbonate, and saturated sodium chloride, and dried (Na_2SO_4). Evaporation *in vacuo* (bath temperature 0 °C) gave an oil (130 mg) which by bulb-to-bulb distillation gave the *deuteriated nitrile* (93 mg, 70%) (Found: M^+, 124.110 4. $C_8{}^1H_{12}{}^2H_1N$ requires *M*, 124.111 1); δ (CCl_4) 5.45 (2 H, m, *cis*-CH=CH), 2.35 (3 H, m, 2-H and 3-H_2), 2.03 (2 H, dt, *J* 6.5 Hz, 6-H_2), 1.4 (2 H, m, 7-H_2), and 0.91 (3 H, t, *J* 6.5 Hz, *Me*CH_2).

(2S)-(Z)-[2-$^2H_1$]*Oct*-4-*enamide* (12a).—A solution of 2N-sodium hydroxide (0.8 ml) in 30% hydrogen peroxide (10 ml) was added to a mixture of the foregoing nitrile (86 mg, 0.71 mmol) and α-pinene (0.5 ml) in ethanol (2.0 ml). The mixture was stirred for 3.5 h at 45—55 °C, cooled, diluted with saturated sodium chloride (60 ml), and extracted with ethyl acetate (1 × 50, 3 × 20 ml). The organic extract was washed with water, saturated iron(II) sulphate (3 × 20 ml), and saturated sodium chloride, dried, and evaporated to an oil (310 mg). Chromatography on Malinkrodt CC-7 silica gel in dichloromethane gave α-pinene (210 mg) initially, and elution with methanol–dichloromethane (1 : 9) gave a solid (80 mg), m.p. 73—75 °C, which crystallised from ether–pentane to yield (2S)-(Z)-[2-$^2H_1$]*oct*-4-*enamide* (64 mg, 63%), m.p. 77—78 °C (Found: M^+, 142.1215. $C_8{}^1H_{14}{}^2H_1NO$ requires *M*, 142.1 216), δ 6.4—5.1 (2 H, $CONH_2$), 5.40 (2 H, m, *cis*-CH=CH), 2.3 (3 H, m, 2-H and 3-H_2), 2.0 (2 H, dt, *J* 6.5 Hz, 6-H_2), 1.4 (2 H, m, 7-H_2), and 0.89 (3 H, t, *J* 7 Hz, *Me*CH_2); *m/e* 142 (M^+, 100%), 113 (35), 99 (25), 73 (25), 72 (20), and 60 (90) [141/142 <3%].

A parallel preparation from the unsaturated acetal (5) in the unlabelled series gave (Z)-*oct*-4-*enamide*, m.p. 78—78.5 °C (Found: C, 67.0; H, 12.0; N, 9.7. $C_8H_{15}NO$ requires C, 67.2; H, 11.9; N, 9.8%); n.m.r. spectrum as above except that the signal at δ 2.3 corresponded to 4 H; *m/e* 141 (M^+, 100%), 112 (40), 98 (25), 72 (40), and 59 (90) [140/141 <2%].

(2S)-(Z)-[2-$^2H_1$]*Oct*-4-*enoic Acid* (16).—The above amide (61 mg, 0.43 mmol) was added to 0.5N-sulphuric acid (20 ml) and the mixture was heated under reflux for 3.5 h, cooled, saturated with sodium chloride, and extracted with chloroform (4 × 10 ml). The organic extract was washed with saturated sodium hydrogen carbonate (3 × 10 ml), the combined alkaline washings were acidified with 10N-hydrochloric acid, and the mixture was saturated with sodium chloride and extracted with chloroform (4 × 10 ml). The chloroform extract was washed with saturated sodium chloride, dried, and evaporated to give the *deuteriated acid* (31 mg, 51%) as a colourless oil (Found: M^+, 143.1058. $C_8{}^1H_{13}{}^2H_1O_2$ requires *M*, 143.1056); δ 10.0 (1 H, br s, CO_2H), 5.4 (2 H, m, *cis*-CH=CH), 2.35 (3 H, m, 2-H and 3-H_2), 2.0 (2 H, dt, *J* 6.5 Hz, 6-H_2), 1.35 (2 H, m, 7-H_2), and 0.89 (3 H, t, *J* 7 Hz, *Me*CH_2).

Degradation of (2S)-(Z)-[2-$^2H_1$]*Oct*-4-*enoic Acid* (16) *to* (+)-(2S)-[2-$^2H_1$]*Succinic Acid* (17).—Ozonised oxygen (*ca.* 5%) was passed for 4 h through a solution of the foregoing acid (16) (28 mg) in dichloromethane (20 ml) at −78 °C. Excess of ozone was then removed in a stream of nitrogen, the solution was evaporated *in vacuo*, and the residue was heated under reflux with 30% hydrogen peroxide (1 ml), formic acid (1 ml), and water (3 ml); more hydrogen peroxide (*ca.* 0.5 ml) was added periodically. The residue from evaporation was washed with chloroform and the insoluble solid was sublimed at 105 °C and 0.07 mmHg (yield 11 mg). This was recrystallised three times from ethyl acetate to give (+)-(2S)-[2-$^2H_1$]succinic acid (6.4 mg), m.p. 186—186.5 °C (lit.,[28] 184—186 °C); the deuteriated acid showed no u.v. absorbtion >250 nm. Mass-spectrometric measurements [16] (average of five determinations) showed that the succinic acid had 0.95 ± 0.03 deuterium atoms per molecule. O.r.d. measurements [15] (Table 3) were performed on the acid (3.245 mg) in water (240 mg); the average of rotations over the range 232—323 nm was 84% of standard, corresponding (after allowance for 95% $^2H_1$-species) to 88% of standard, *i.e.* 94% of (+)-(2S)-acid and 6% of (−)-(2R)-acid. A reasonable estimate of the accuracy is ±5%.

TABLE 3

O.r.d. measurements on the succinic acid

λ/nm	Standard [15] [α] (°)	Found [α] (°)	% of Standard
232	52.4	+48.0	92
238	37.5	+34.1	91
244	26.2	+23.8	91
250	18.2	+16.6	91
256	13.0	+12.0	92
263	10.1	+8.98	89
270	7.87	+6.98	89
278	6.25	+5.52	88
286	5.07	+4.23	83
294	4.13	+3.23	78
303	3.35	+2.61	78
313	2.74	+2.18	80
323	2.25	+1.62	72

Conversion of (Z)-*Oct*-4-*enamide* (12) *into* 1-*Aminoheptane* (14).—Unlabelled (Z)-oct-4-enamide [as (12)] (300 mg, 2.1 mmol) in ethyl acetate (30 ml) was hydrogenated at 1 atm and 20 °C over Adams catalyst (20 mg) (uptake 1.05 mol equiv. over 1 h). The solution was filtered (Celite) and evaporated to a solid, which crystallised from ethyl acetate–light petroleum to give octanamide [as (13)] (280 mg, 93%), m.p. and mixed m.p. with authentic material 104—105 °C (lit.,[27] 105 °C) (Found: C, 67.0; H, 12.0; N, 9.7. $C_8H_{17}NO$ requires C, 67.0; H, 12.0; N, 9.8%).

The octanamide (250 mg, 1.75 mmol) was boiled with 0.5N-sulphuric acid as before to give octanoic acid [as (14)] ($\nu_{max.}$ 3 400—2 300, 1 700, and 920 cm^{-1}), which was treated immediately with chloroform (5 ml) and concentrated sulphuric acid (1.0 ml). The stirred mixture was warmed to 50 °C, sodium azide (115 mg, 1.77 mmol) was added in

small portions during 45 min, and stirring was continued for 3 h at 50 °C. The cooled mixture was diluted with ice–water (100 ml) and washed with chloroform (2 × 20 ml); the aqueous solution was brought to pH 11 with 2N-sodium hydroxide and was extracted with dichloromethane. The organic extract was washed with saturated sodium chloride, dried (K_2CO_3), and evaporated under reduced pressure (bath temperature 20 °C) to give 1-aminoheptane (200 mg, 100%), δ 2.64 (2 H, br t, 1-H_2), 1.3 (10 H, m, $[CH_2]_5$), 1.16 (2 H, s, NH_2), and 0.86 (3 H, distorted t, $MeCH_2$).

For characterisation, the amine (20 mg) in 10% aqueous acetic acid (0.2 ml) was treated with a warm solution of sodium cyanate (15 mg) in water (0.2 ml), and the solution was heated on the steam-bath for 0.5 h and then cooled. The precipitated solid crystallised from chloroform to give *N*-heptylurea (16 mg), m.p. and mixed m.p. with authentic material 112—112.5 °C (lit.,[27] 111 °C) (Found: C, 60.8; H, 11.2; N, 17.2. $C_8H_{18}N_2O$ requires C, 60.7; H, 11.5; N, 17.7%); $\nu_{max.}$ (Nujol): 3 370, 3 190, 1 655, and 1 600 cm^{-1}; δ[$CDCl_3$–C_5D_5N (2 : 1)] 5.2 (2 H, br s, NH_2), 4.0 (1 H, s, NH), 3.15 (2 H, m, 1-H_2), 1.6—1.0 (10 H, m, $[CH_2]_5$), and 0.84 (3 H, distorted t, $MeCH_2$).

1-Aminoheptane was stored as the stable picrate made by treating the amine (100 mg, 0.870 mmol) in ethanol (1 ml) with dry picric acid (200 mg, 0.872 mmol) in hot ethanol (*ca.* 5 ml). It was recrystallised twice from chloroform to give 1-aminoheptane picrate (270 mg, 90%), m.p. and mixed m.p. with authentic material 117—118 °C (lit.,[27] 120—122 °C). The free amine was obtained by passing a solution of the picrate in ethanol–chloroform (1 : 9) down a column of alumina (Spence type H; 1 : 100) followed by evaporation of the solvent under a stream of nitrogen.

Synthesis of Labelled 1-*Aminoheptanes*.—(a) 1-*Amino*-[1-^{14}C]*heptane*. Hexan-1-ol (2.0 g, 19.6 mmol) was treated with toluene-4-sulphonyl chloride (4.1 g, 21.5 mmol) in pyridine (20 ml) as before to give, finally, hexyl toluene-4-sulphonate (19) 4.65 g, 91%), homogeneous on t.l.c. (SiO_2, C_6H_6, R_F 0.45); δ (CCl_4) 7.63 and 7.24 (4 H, d + d, AA'BB' pattern, aryl-H), 3.90 (2 H, t, *J* 6.5 Hz, CH_2), 2.41 (3 H, s, aryl-Me), 1.9—1.0 (8 H, m, $[CH_2]_4$), and 0.84 (3 H, distorted t, $MeCH_2$).

Unlabelled sodium cyanide (5 mg, 0.1 mmol) was added to a solution of the toluene-4-sulphonate (19) (500 mg, 1.95 mmol) in dry dimethylformamide (20 ml) at 20 °C under nitrogen, and the mixture was stirred for 2 h by which time all the solid had dissolved. Sodium [^{14}C]cyanide (*ca.* 0.5 mg, 0.01 mmol; 500 μCi) (Radiochemical Centre, Amersham) was added and after a further 18 h an excess of unlabelled sodium cyanide (500 mg, 10.2 mmol) was added and stirring was continued for another 4 h. The mixture was diluted with unlabelled heptanenitrile (500 mg) and was worked up as above to give an oil which after bulb-to-bulb distillation afforded [1-^{14}C]heptanenitrile (20) (672 mg, 80%; 170 μCi); $\nu_{max.}$ 2 250 cm^{-1}; δ (CCl_4) 2.25 (2 H, distorted t, 2-H_2), 1.9—1.1 (8 H, m, $[CH_2]_4$), and 0.91 (3 H, distorted t, $MeCH_2$).

The foregoing nitrile (20) (35 mg, 0.315 mmol; 9.85 μCi) in ethanol (5 ml) was added to a solution of heptanenitrile (600 mg, 5.4 mmol) and 5N-hydrochloric acid (2.5 ml) in ethanol (20 ml) and the resulting solution was hydrogenated for 16 h at 1 atm and 20 °C over 10% palladium–charcoal (0.5 g) (uptake 2.1 mol equiv.). The solution was filtered (Celite), concentrated to *ca.* 5 ml, diluted with water, and washed with ether. The aqueous solution was brought to pH 11 with 2N-sodium hydroxide, and was worked up as for the unlabelled material to give 1-amino[1-^{14}C]heptane (15) (540 mg, 85%; 7.6 μCi), characterised as the urea (1.6 μCi $mmol^{-1}$), m.p. and mixed m.p. 111—112 °C. The amine (500 mg) gave the [^{14}C]picrate (1.34 g, 90%), m.p. and mixed m.p. 117—118 °C.

(b) (1RS)-1-*Amino*[1-$^3H_1$]*heptane* (15b *and* c). 0.1N-Sodium hydroxide (0.2 ml) and sodium borohydride (5 mg, 0.13 mmol) were added in turn to a stirred solution of (*Z*)-hept-3-enal (7) (1.0 g, 8.95 mmol) in ethanol (20 ml) under nitrogen at 20 °C. After 0.5 h, Na_3BH_3T (*ca.* 3.2 mg, 0.085 mmol; 50 mCi) (Radiochemical Centre, Amersham) was added, and the mixture was stirred for a further 18 h. Excess of unlabelled sodium borohydride (100 mg) was added, and after 1 h, the mixture was diluted with half-saturated sodium chloride (150 ml). Unlabelled (*Z*)-hept-3-en-1-ol (0.2 g, 1.79 mmol) was added, the unreacted sodium borohydride was decomposed with 3N-sulphuric acid, and the mixture was extracted with ether (5 × 30 ml). The combined ether extracts were washed with saturated sodium hydrogen carbonate and saturated sodium chloride, dried, and evaporated to give an oil (970 mg). Bulb-to-bulb distillation gave (1*RS*)-(*Z*)-[1-$^3H_1$]hept-3-en-1-ol (8b and c) (690 mg, 57%; 27 mCi), characterised as the biphenyl-4-ylurethane (4.47 mCi $mmol^{-1}$), m.p. and mixed 84—85 °C.

The foregoing alcohol (8b and c) (160 mg, 1.4 mmol; 6.26 mCi) was converted into (2*RS*)-(*Z*)-[2-$^3H_1$]oct-4-enamide (12b and c) (69 mg, 0.49 mmol; 2.18 mCi), m.p. and mixed m.p. 77—78 °C, exactly as in the deuteriated series. As described for the unlabelled series, the (*RS*)-amide (65 mg, 0.46 mmol; 2.06 mCi) was converted into (1*RS*)-1-amino[1-$^3H_1$]heptane (*ca.* 45 mg, 85%) which was diluted with 1-aminoheptane (250 mg) and distilled bulb-to-bulb (90—95 °C at 25 mmHg) to give (1*RS*)-1-amino[1-$^3H_1$]heptane (15b and c) (255 mg, 2.22 mmol; 1.70 mCi), characterised as its urea (800 μCi $mmol^{-1}$), m.p. and mixed m.p. 111—112 °C.

(c) (1S)-1-*Amino*[1-$^3H_4$]*heptane* (15b). An aqueous solution of (1*RS*)-[1-$^3H_1$]ethanol was prepared as follows. 0.1N-Sodium hydroxide (0.2 ml) and sodium borohydride (1.5 mg, 0.04 mmol) were added in turn to a stirred solution of acetaldehyde (44 mg, 1.0 mmol) in water (2 ml) under nitrogen. After 5 min sodium [$^3H_4$]borohydride (*ca.* 3.2 mg, 0.085 mmol; 50 mCi) was added with 0.1N-sodium hydroxide (5 ml) and the solution was stirred for 16 h at 20 °C under nitrogen. Then sodium borohydride (30 mg) was added, and after 1 h, the unreacted borohydride was decomposed with 3N-sulphuric acid (to pH 1). The volatile materials were distilled off in a vacuum-transfer apparatus (0.04 mmHg, bath temperature 20 °C) to give (1*RS*)-[1-$^3H_1$]ethanol in water (7 ml).

Albumin (40 mg) and NAD^+ (20 mg; free acid) were dissolved in a solution containing 0.2M-phosphate (K^+) buffer, pH 7.0 (25 ml), distilled water (80 ml), and the foregoing tritiated ethanol (*ca.* 50 mCi). This solution was added to (*Z*)-hept-3-enal (7) (250 mg, 2.23 mmol) in dioxan (2 ml). The solution was cleared by warming to 30 °C, ethanol-free horse-liver alcohol dehydrogenase (5 mg; *ca.* 1.4 units mg^{-1}) was added and the solution was kept under nitrogen at 30 °C in the dark; further additions of the ethanol-free enzyme (10 mg) and NAD^+ (10 mg) were made at 16-h intervals. After 48 h, the reaction mixture contained the starting aldehyde and the required alcohol in the ratio 7 : 1 by g.l.c. (column II, 110 °C, retention times 4.2 and 10.1 min, respectively). Ethanol (2 g), liver alcohol

dehydrogenase (5 mg), and NAD^+ (10 mg) were then added. After incubation at 30 °C for a further 24 h the reduction was essentially complete (g.l.c.). The reaction mixture was diluted with (*Z*)-hept-3-en-1-ol (8) (400 mg) and worked up as for the deuteriated series to give (1*R*)-(*Z*)-[1-$^3H_1$]hept-3-en-1-ol (8b) (435 mg, 3.81 mmol; 1.95 mCi), characterised as the biphenyl-4-ylurethane (513 μCi $mmol^{-1}$), m.p. and mixed m.p. 84—85 °C.

The (1*R*)-alcohol (8b) (400 mg, 3.5 mmol; 1.8 mCi) was converted into (2*S*)-(*Z*)-[2-$^3H_1$]oct-4-enamide (12b) (180 mg, 1.28 mmol; 656 μCi), m.p. and mixed m.p. 77—78 °C, and thence as above (without dilution) into (1*S*)-1-amino[1-$^3H_1$]-heptane (15b) (130 mg, 1.13 mmol, 580 μCi), characterised as the urea (513 μCi $mmol^{-1}$), m.p. and mixed m.p. 111—112 °C.

(d) (1R)-1-*Amino*[1-$^3H_1$]*heptane* (15c). A stirred solution of chromium trioxide–pyridine complex was prepared *in situ* [18] from chromium trioxide (1.8 g, 18 mmol) and pyridine (3 ml, 36 mmol) in dry dichloromethane (45 ml) at 20 °C. After 15 min, (1*RS*)-(*Z*)-hept-3-en-1-ol (8b and c) (240 mg, 2.1 mmol, 9.4 mCi) was added and stirring was continued for 20 min. The mixture was diluted with ether (100 ml), washed with 1N-sodium hydroxide (4 × 50 ml), 2N-hydrochloric acid (2 × 20 ml), saturated sodium hydrogen carbonate, and saturated sodium chloride, dried, and evaporated (bath temperature 20 °C) to give the aldehyde (9) as an oil (*ca.* 200 mg, 83%), free from unreacted alcohol by t.l.c. [SiO_2, chloroform–benzene (1 : 1), R_F 0.6].

The foregoing [^3H-*formyl*]aldehyde (9) was distilled bulb-to-bulb and the colourless oil was reduced immediately as in (c) above, with the following differences: *ca.* 3 ml of *unlabelled* ethanol was used in place of the [$^3H_1$]ethanol; the enzymic reduction was essentially complete after 48 h (g.l.c.). The (1*S*)-(*Z*)-[1-$^3H_1$]hept-3-en-1-ol (8c) (300 mg, 2.7 mmol; 157 μCi) was diluted with unlabelled material (to 450 mg) and converted as for the (1*R*)-alcohol into (1*R*)-1-amino[1-$^3H_1$]heptane (15c) (145 mg, 1.27 mmol; 52.6 μCi), characterised as the urea (41.4 μCi $mmol^{-1}$), m.p. and mixed m.p. 111—112 °C. This (1*R*)-amine (140 mg) was stored as its picrate (360 mg, 90%).

Enzymic Conversions with Monoamine Oxidase from Rat Liver.—Mitochondria were prepared from rat-liver homogenates as described previously,[29a] and then frozen for at least 48 h. The solubilisation and partial purification of the enzyme were carried out as in ref. 29*b*, and the fractions from the first Sephadex G-200 column which contained active enzyme were pooled and used directly for the enzymic conversions. Activity was measured spectrophotometrically [29c] and is expressed as units ml^{-1}; 1 unit represents the ability to oxidise 1 μmol benzylamine per min at 30 °C. Values found ranged from 3 to 16 units ml^{-1}; the typical quantity obtained in each preparation from livers of 20—25 rats was *ca.* 40 ml, with an average protein concentration of *ca.* 15 mg ml^{-1}.

(a) *With tritiated substrates.* The tritiated sample of 1-aminoheptane (*ca.* 57 mg, 0.5 mmol) in 0.2M-phosphate (K^+) buffer, pH 7.0 (10 ml) was added to a gently agitated solution containing the monoamine oxidase preparation (*ca.* 20 ml, 80—160 units), 70mM-phosphate buffer, pH 7.0 (40 ml), ethanol (4 ml), NAD^+ (20 mg; free acid, Boehringer), catalase (E.C. 1.11.1.6.) (*ca.* 500 units; specific activity *ca.* 50 units μg^{-1}, Boehringer), and horse-liver alcohol dehydrogenase (10 mg; specific activity *ca.* 2.7 units mg^{-1}, Boehringer). The reaction mixture was kept at 30 °C under oxygen in the dark; further alcohol dehydrogenase (5 mg), NAD^+ (5 mg), and catalase (*ca.* 100 units), each in 70mM-phosphate buffer, pH 7.0 (0.5 ml), were added *via* a syringe every 2 h for a total reaction time of 20 h. The mixture was cooled, acidified with 5N-sulphuric acid (to pH 1), saturated with sodium chloride, and extracted continuously with ether for 10 h. The ether extract was dried (Na_2SO_4) and concentrated (bath temperature 30 °C), and the residue was distilled bulb-to-bulb (*ca.* 100 °C and 15 mmHg) to give the tritiated heptan-1-ol. Its biphenyl-4-ylurethane derivative was prepared as before, was purified by p.l.c. on silica gel [chloroform–benzene (3 : 5), R_F 0.5], and recrystallised from ligroin to constant specific activity; m.p. 105—106 °C (lit.,[11a] 105 °C).

A parallel radio-inactive run gave unlabelled material (Found: C, 77.1; H, 8.1; N, 4.6. $C_{20}H_{25}NO_2$ requires C, 77.2; H, 8.05; N, 4.5%); $\nu_{max.}$ (KBr) 3 340, 1 700, 1 260, and 1 240 cm^{-1}; δ 7.7—7.2 (9 H, m, aryl-H), 6.7 (1 H, br s, NH), 4.1 (2 H, distorted t, 1-H_2), 1.9—1.0 (10 H, m, $[CH_2]_5$), and 0.88 (3 H, distorted t, $MeCH_2$).

(b) *With doubly-labelled substrates.* The appropriate 1-amino[1-^3H]heptane picrate was mixed with the picrate of the ^{14}C-material in proportions which gave a ^3H : ^{14}C ratio of *ca.* 10 : 1 Sufficient unlabelled 1-aminoheptane picrate was added to give a ^{14}C-specific activity in the range (0.5—5) × 10^3 disintegrations min^{-1} mg^{-1} for the free amine, and the picrate mixture was then recrystallised twice from chloroform. The free amine from part of the picrate (*ca.* 60 mg) was recovered as earlier and was converted immediately into its urea; this was recrystallised to constant specific activity and ^3H : ^{14}C ratio.

Initially, the enzymic runs were carried out as described under (a). However, it was found that the competing aldehyde dismutase reaction [25] was partially suppressed when the following conditions were used.

The freshly prepared monoamine oxidase (*ca.* 25 ml, 110—160 units) was added to a gently agitated solution of NAD^+ (20 mg; free acid), horse-liver alcohol dehydrogenase (10 mg; specific activity *ca.* 2.7 units mg^{-1}, Boehringer), ethanol (2 ml), and dioxan (2 ml) in 0.8M-phosphate (K^+) buffer, pH 7.6 (25 ml), and water (50 ml) at 30 °C under oxygen in the dark. The 1-amino[1-^{14}C,1-$^3H_1$]heptane (*ca.* 8 mg), recovered from its picrate (25 mg) as usual, was added every 4 h as a solution in 0.2M-phosphate buffer, pH 7.6 (1 ml) until *ca.* 50 mg of the amine had been added; additions of more alcohol dehydrogenase (5 mg) and NAD^+ (10 mg) were made every 8 h. After a total time of 30 h, the reaction mixture was worked up as under (a) above to give the doubly-labelled biphenyl-4-ylurethane derivative, which was purified as before and then recrystallised to constant specific activity and ^3H : ^{14}C ratio.

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