Glyceryl-ether monooxygenase [EC 1.14.16.5]. Part 9. Stereospecificity of the oxygenase reaction

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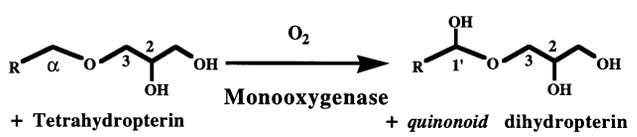
(2*RS*,1′*R*)-[1′-$^{3}H_1$]- and (2*RS*, 1′*S*)-[1′-$^{3}H_1$]-Hexadecyloxypropane-1,2-diols (chimyl alcohols) have been prepared and their stereochemistry has been confirmed by synthesizing the [$^{2}H_1$]-analogues using similar procedures. When they were used as substrates for glyceryl-ether monooxygenase from rat liver in the presence of oxygen and (*RS*)-6-methyl-5,6,7,8-tetrahydropterin as co-factor, the 1′*S*-isomer released 37% of its tritium into the aqueous buffer after 20 mins, whereas the 1′*R*-isomer released only 6.5% showing that the reaction was stereospecific for the *pro*-H_S hydrogen atom of the glyceryl ether substrate. This was in agreement with the kinetic parameters of unlabelled-(2*RS*)-3-, (2*RS*, 1′*R*)-3-[1′-$^{2}H_1$]-, (2*RS*, 1′*S*)-3-[1′-$^{2}H_1$]- and (2*RS*)-3-[1′,1′-$^{2}H_2$]-hexadecyloxypropane-1,2-diols where the apparent K_m values were about the same (49.4, 53.7, 49.3 and 54.0 μM respectively) but the apparent maximum velocities (V_{max} in nmol min^{-1} mg^{-1} protein) of the first two substrates (37.5 and 37.5) were faster than for the latter two substrates (22.5 and 23.6), consistent with the *pro*-H_S hydrogen atom being replaced by the hydroxy group and a primary deuterium isotope effect of ~1.6.

Glyceryl-ether monooxygenase is a mixed function oxidase that has strict requirements for oxygen and a 5,6,7,8-tetrahydropterin in order to hydroxylate the α carbon atom of the aliphatic side chain of 1-alkylglyceryl ethers (Scheme 1).[1,2] It is a



Scheme 1

microsomal enzyme which is widely distributed in mammalian tissues.[3] It is an important enzyme in ether lipid metabolism and although ether lipid levels in liver and blood plasma are low, ether lipids do account for about 19% of total phospholipids in man.[4] The development of a rapid and direct spectrophotometric assay for measuring its activity[2] has made it possible to study the stoichiometry of the enzymic reaction,[5] and the specificity with respect to the pterin co-factor[2] and the ether lipid substrates.[6] With this assay where the membrane-bound enzyme and the lipid substrates are solubilised by the non-ionic detergent, Mega-10, the kinetic parameters (K_m and V_{max}) for both the three pterin co-factors and a variety of lipid substrates were determined with the usual degree of accuracy.[2,6] The natural pterin co-factor, by analogy with the phenylalanine monooxygenase system,[7] is most probably (6*R*)-5,6,7,8-tetrahydro-*erythro*-biopterin and its larger *V*/*K* value compared with the non-natural co-factors 6-methyl- and *cis*-6,7-dimethyl-5,6,7,8-tetrahydropterin is consistent with its being a better co-factor.[2] The enzymic activities for (6*R*)- and (6*S*)-6-methyl-5,6,7,8-tetrahydropterin show that they are almost equally effective as co-factors for glyceryl-ether monooxygenase.[5] Similarly, enzyme activity is not sensitive to the configuration at C-2 of 1-glyceryl ether substrates, *i.e.* (2*R*)-3-hexadecyloxypropane-1,2-diol (natural chimyl alcohol) and (2*R*)-3-octadecyloxypropane-1,2-diol (natural batyl alcohol) are almost as active as their respective 2*S* enantiomers.[6] We present here evidence that the stereospecificity at the reaction centre, on the other hand, has a strong bias for the *pro-S* hydrogen atom at C-1′ of the alkyl side-chain which is displaced in preference to the *pro-R* hydrogen in the oxygenase reaction.

Stereochemistry

We have synthesized (1′*S*)- and (1′*R*)-3-[1′-$^{3}H_1$]hexadecyloxypropane-1,2-diols and subjected them to the enzymic reaction using (*RS*)-6-methyl-5,6,7,8-tetrahydropterin as co-factor. During the hydroxylation hydrogen or tritium is released (depending on the stereospecificity) as water or tritiated water, respectively. Aliquots of the reaction mixture were distilled *in vacuo* and the radioactivity in the aqueous distillate was measured. The data showed that increasing amounts of tritiated water were released from the (1′*S*)-propanediol with time and accounted for 37% of the radioactivity after 20 mins, whereas only 6.5% of the radioactivity was released from the (1′*R*)-propanediol (Fig. 1). In order to obtain these results it was necessary to increase the amount of enzyme seven-fold compared with the standard assay conditions, but in addition human dihydropteridine reductase and NADH were included so as to recycle the pterin co-factor because of the large tritium isotope effect. With less enzyme, and without recycling of the co-factor, tritium release was very slow although much of the non-labelled substrate was consumed, and the activity of the enzyme deteriorated over long periods of time. The small release of tritium from the 1′*R*-isomer in the modified reaction was most probably due to the presence of some of the 1′*S*-isomer in the sample. The ^{1}H NMR data of the camphanate esters of the respective precursor chiral monodeuterohexadecanols (see below) showed that the (1′*R*)-propanediol should be indeed contaminated with 5–10% of the 1′*S*-enantiomer and is consistent with the results in Fig. 1. Since 37% of tritium label from the (1′*S*)-propanediol in the presence of some (1′*R*)-propanediol (~5–10%) was released after 20 mins then a large amount of unlabelled substrate, compared with labelled substrate, must have been consumed as a result of the tritium iso-

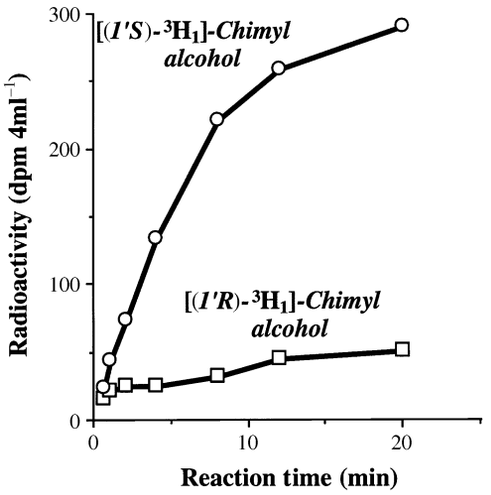
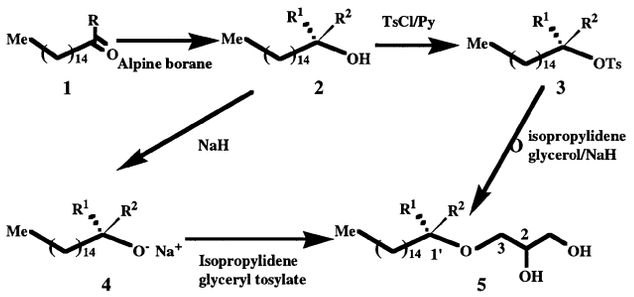


Fig. 1 Tritium release from [(1′*S*)-$^3H_1$]- and [(1′*R*)-$^3H_1$]-3-hexadecyloxypropane-1,2-diols by glyceryl-ether monooxygenase

tope effect,[8] which must be larger than the deuterium isotope effect observed (see kinetic data below). After 20 mins the reaction will have slowed down considerably due to the large decrease in the substrate concentration (> 60%) as well as due to enzyme inactivation which is known to occur after this time. In order to establish the stereochemistry of the mono-tritiated chimyl alcohols the respective mono-deuteriated chimyl alcohols had to be synthesized using the same reactions (see syntheses below).

The stereospecificity observed with the tritiated substrates should also be consistent with the enzymic activities of the related chiral deuteriated analogues because the C–D bond is more difficult to break than the C–H bond, and the enzymic reaction necessarily results in the cleavage of a C–H or a C–D bond. The difference of the cleavage between the two bonds should show up in the maximum velocities of the optical isomers. The kinetic parameters using the standard assay procedure were measured, with (*RS*)-6-methyl-5,6,7,8-tetrahydropterin as co-factor, for unlabelled, mono-deuteriated (1′*S*)- and (1′*R*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diols, as well as the dideuteriated [1′,1′-$^2H_2$]hexadecyloxypropane-1,2-diol. All compounds were racemic at C-2. The kinetic parameters were as follows: 3-hexadecyloxypropane-1,2-diol had K_m 49.4 (± 12.6) μM and V_{max} 37.5 (± 4.5 nmol min^{-1} mg^{-1} protein), (1′*R*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diol had K_m 53.7 (± 9.4) μM and V_{max} 37.5 (± 2.5 nmol min^{-1} mg^{-1} protein), (1′*S*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diol had K_m 49.3 (± 11.5) μM and V_{max} 22.5 (± 2.4 nmol min^{-1} mg^{-1} protein) and 3-[1′,1′-$^2H_2$]hexadecyloxypropane-1,2-diol had K_m 54.0 (± 11.7) μM and V_{max} 23.6 ± 2.3 nmol min^{-1} mg^{-1} protein). It is clear that while the K_m values are similar, the maximum velocities of the 1′*S* mono-deuteriated alcohol and the 1′,1′-dideuteriated alcohol are similar and are ~37% slower than the values of the 1′*R* mono-deuteriated alcohol and the unlabelled alcohol which have the same velocities. There is a deuterium isotope effect of 1.6 for the (1′*S*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diol consistent with a primary isotope effect, and a stereoselectivity for the *pro-S* hydrogen atom as observed with the tritiated alkoxypropanediol above.

These data tell us that the monooxygenase reaction is stereospecific but they do not show whether the hydroxylation proceeded with retention or with inversion of configuration at C-1′ of the ether lipid side-chain. We have been unable so far to isolate the intermediate chiral hemiacetal in order to attempt to determine this point. However, it appears to be generally the case with oxygenations of methylene groups in fatty acid and sterol metabolism by mixed function oxidases where hydroxylations occur stereospecifically and with *retention* of configuration.[9–14] By analogy, we presume that hydroxylation of the 1′-methylene group in the alkyl chain of 3-alkoxypropanediols by this monooxygenase takes place with retention of configuration.



Syntheses

(1*R*)-(**2**; R^1 = H, R^2 = ^{2}H) and (1*S*)-(**2**; R^1 = ^{2}H, R^2 = H) [1′-$^2H_1$]hexadecanols were prepared by stereospecific reduction of [1-$^2H_1$]hexadecanal **1** (R = ^{2}H) {obtained by reduction of ethyl hexadecanoate with lithium aluminium deuteride followed by oxidation of the [1,1-$^2H_2$]hexadecanol **2** (R^1 = R^2 = ^{2}H) formed with pyridine–chromium trioxide complex[15]} with (*S*)- and (*R*)-Alpine-boranes, respectively, which are known to be highly stereoselective reducing agents.[16–18] The absolute configurations of the hexadecanols were confirmed by their respective plain positive (for the *R*-isomer) and plain negative (for the *S*-isomer) ORD curves as observed previously with various *R* and *S* [1-$^2H_1$]alcohols.[19] Moreover the ^{1}H NMR spectra of the (−)-camphanoyl esters of the alcohols **2** (R^1 = H, R^2 = ^{2}H) and **2** (R^1 = ^{2}H, R^2 = H) in hexadeuteriobenzene in the presence of 1.2 mol of Eu(dpm)$_3$ and decoupling of the 2′-methylene protons showed that the resonance signal of the 1′-hydrogen atom (equivalent to *pro-S*) of the ester from the (*R*)-alcohol with the 1′-H was a singlet at lower field (δ 4.09 ppm) than the 1′-hydrogen atom (equivalent to *pro-R*) of the ester from the (*S*)-alcohol (δ 4.06 ppm); as was previously observed with (−)-camphanoyl esters of chiral 1-monodeuteriated alcohols.[18–20] The relative shifts of these signals were confirmed by mixing various amounts of the labelled esters as well as various amounts of the unlabelled ester to these mixtures and observing the signal heights and measuring the areas under the peaks. These indicated that the (*R*)- and (*S*)-[1-$^2H_1$]hexadecanols were of 90–95% stereochemical purity as was reported previously for other chiral monodeuteriated alcohols prepared by similar reductions,[18,20–22] particularly when straight chain aldehydes were reduced. The mass spectra (EI) of these deuteriohexadecanols gave weak molecular ion peaks. However, the M − $H_2O^{\cdot+}$ peaks were intense and, when compared with unlabelled hexadecanol, were used to determine the deuterium content. These showed that 1–1.5% of the unlabelled alcohol was present in the monodeuteriohexadecanols. The 1,1-dideuteriohexadecanol behaved similarly and showed minor contamination with monodeuteriohexadecanol (~3%) and unlabelled alcohol (~2%).

The hexadecyloxypropanediols **5** (R^1 = H, R^2 = ^{2}H), **5** (R^1 = ^{2}H, R^2 = H), **5** (R^1 = H, R^2 = ^{3}H), **5** (R^1 = ^{3}H, R^2 = H), **5** (R^1 = R^2 = H) and **5** (R^1 = R^2 = ^{2}H), were prepared by reactions of the respective hexadecyl tosylates **3** (R^1 = ^{2}H, R^2 = H), **3** (R^1 = H, R^2 = ^{2}H), **3** (R^1 = ^{3}H, R^2 = H), **3** (R^1 = H, R^2 = ^{3}H), **3** (R^1 = R^2 = H) and **3** (R^1 = R^2 = ^{2}H) with isopropylideneglycerol sodium salt in dimethylformamide followed by acid hydrolysis, a procedure which was successfully used for preparing several-1-alkyl glyceryl ethers before.[6] The configurations at C-1′ of the chiral diols **5** was assumed to be opposite to those present in the respective chiral hexadecanols **2** because the reaction of the isopropylidene anion with hexadecyl tosylate was taken to occur with inversion of configuration at the carbon atom bearing the tosylate group. This was shown to be true in this case by carrying out the reaction between isopropylidene tosylate sep-

arately with the sodium hexadecylates **4** (R^1 = H, R^2 = ^{2}H) and **4** (R^1 = ^{2}H, R^2 = H), which after acid hydrolysis, provided the chiral diols **5** (R^1 = H, R^2 = ^{2}H) and **5** (R^1 = ^{2}H, R^2 = H) respectively with retention of configuration at C-1′ because it was *not* the reaction centre. Thus the [(1′*R*)-$^2H_1$]-diol **5** (R^1 = H, R^2 = ^{2}H) derived from the tosylate **3** {[(1*S*)-$^2H_1$], R^1 = ^{2}H, R^2 = H)} had the same plain positive ORD curve as the diol derived from the sodium hexadecylate **4** {[(1*R*)-$^2H_1$], R^1 = H, R^2 = ^{2}H} and isopropylidene glyceryl tosylate. Similarly, the [(1′*S*)-$^2H_1$]-diol **5** (R^1 = ^{2}H, R^2 = H) from the [(1*R*)-$^2H_1$]hexadecyl tosylate **3** (R^1 = H, R^2 = ^{2}H) has the same plain negative ORD curve as the diol derived from the sodium hexadecylate **4** {[(1*S*)-$^2H_1$], R^1 = ^{2}H, R^2 = H} and isopropylidene glyceryl tosylate. The mass spectra as above of these monodeuteriopropane-1,2-diols also gave weak molecular ion peaks. On the other hand, the M − $CH_3O^{\bullet+}$ peaks were intense and when compared with the unlabelled diol showed that the 1′*R* and 1′*S* isomers contained ~7–8% of unlabelled diol. It is difficult to explain the presence of these amounts of unlabelled diol (see hexadecanol precursors above), unless there was some amount of scrambling between the M − $CH_3O^{\bullet+}$ and $CH_3^{\bullet+}$ ions in the spectrometer.

Experimental

The purity of all compounds was confirmed by TLC in two systems: (A) Merck aluminium oxide 60 F_{254}, Type E plates eluted with solvent (a) CH_2Cl_2 or solvent (b) CH_2Cl_2–MeOH (10:1) and (B) Merck Kieselgel 60 F_{254} plates eluted with solvents (a) or (b), and visualised with iodine vapour, as well as by elemental analyses of compounds where the melting points are stated (The Australian National University Microanalytical Services). ^{1}H NMR spectra were run at 300 MHz (Varian Gemini 300, or Varian VXR 300) in $CDCl_3$ with Me_4Si as internal standard unless otherwise stated, chemical shifts (δ) are in ppm and *J* values are in Hz. IR spectra (KBr) were measured on a Perkin-Elmer 1600 Series FTIR. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter at 25 °C using mercury light. UV spectra were measured on Cary (Varian) 118 and 219 spectrometers with 1 cm masked cuvettes thermostatted at 25 ± 0.5 °C. Mass spectra (Fisons Instruments, AutoSpec, mass spectrometer operating at 70eV) of key deuteriated compounds were determined and compared with non-labelled compounds to obtain the amount of deuteriation (only relevant peaks are reported).

[1,1-$^2H_2$]Hexadecanol

To a suspension of lithium aluminium deuteride (1 g, 24 mmol) in dry ether (50 ml) was added, with stirring over 1 h, a solution of ethyl hexadecanoate (6 g, 21 mmol) in dry ether (25 ml) under N_2 at 25 °C. Deuterium oxide (4 ml) was added to the cooled mixture which was then stirred at 25 °C for 1 h. Insoluble material was filtered off. The filter cake was washed with ether (50 ml × 2) and the combined ethereal solutions were dried (K_2CO_3) and evaporated to dryness. The residue was recrystallised from hexane to give the *dideuteriohexadecanol*, mp 37–40 °C (4.4 g, 86%); *m*/*z* (rel. intensity) 226.3 (M − $H_2O^{\bullet+}$, 1.0); 225.3 (M − 1 − $H_2O^{\bullet+}$, 0.03) and 224.5 (M − 2 − $H_2O^{\bullet+}$, 0.02) (Found: C, 78.33; H + D, 14.96. $C_{16}H_{32}D_2O$ requires C, 78.61, H + D, 14.83%). [1,1-$^2H_2$]Hexadecyl tosylate (90% yield) was prepared from the alcohol (1 mol) and tosyl chloride (1.2 mol) in pyridine as before[6] and recrystallised from hexane, mp 48–48.5 °C (Found: C, 69.37; H + D, 10.85; S, 7.81. $C_{23}H_{38}D_2O_3S$ requires C, 69.13; H + D, 10.83; S, 8.02%); TLC: R_F (A) 0.20 (a) and 0.87 (b), and (B) 0.37 and 0.95 (b).

[1-$^2H_1$]Hexadecanal

The procedure of Corey and Schmidt[15] was adopted. A mixture of [1,1-$^2H_2$]hexadecanol (4.88 g, 20 mmol) and CrO_3–pyridine (10.7 g, 60 mmol) in CH_2Cl_2 (500 ml) was stirred at 25 °C for 7 days (24 h was sufficient for the non-deuteriated alcohol as checked by TLC on silica gel using CH_2Cl_2 as eluent) and evaporated *in vacuo*. Ether (500 ml) was added to the mixture which was then stirred at 25 °C for 30 min. Insoluble material was filtered off and the filtrate was evaporated *in vacuo*. The residue in CH_2Cl_2 was chromatographed through a Silica Gel 60 column (Merck 0.063–0.20 mesh, 400 g) and eluted with CH_2Cl_2 and recrystallised from 10 parts of methanol to give [1-$^2H_1$]hexadecanal (3.44 g, 71%), mp 35–36 °C (Found: C, 79.57; H + D, 14.07. $C_{16}H_{31}DO$ requires C, 79.60; H + D, 13.77%); TLC: R_F (A) 0.71 (a) and (B) 0.99 (b); ν/cm^{-1} 2941s, 2848s, 2104m (C*D*O), 1703s, 1471s, 1406m, 1098m, 717m and 682w [the respective bands in the non-deuteriated aldehyde were at 2915s, 2860s, 2850s (shoulder), 2700m, 1712s, 1472s, 1410m, 1068w, 717m and 698w]; δ_H C*H*O peak at 9.74 (t, *J* 1.9) in non-deuteriated hexadecanal is absent and the sextet at 2.40 (*J* 1.9 and 7.4) in the non-deuteriated aldehyde is replaced by a triplet at 2.40 (*J* 7.4); *m*/*z* 241.2 M$^{\bullet+}$; and 1′,1′-dideuterohexadecyl hexadecanoate (0.43 g, 9%) had mp 50–51 °C (Found: C, 79.40; H + D, 13.84. $C_{23}H_{62}D_2O_2$ requires C, 79.60; H + D, 13.87%); TLC: R_F (A) 0.95 (a); ν/cm^{-1} 1733s (ester CO), 1169s (COC) and 946m (C$D_2$$CO_2$); and the non-deuteriated ester which was similarly obtained had mp 50–51 °C (Found: C, 80.03; H + D, 13.29. $C_{23}H_{64}O_2$ requires C, 79.93; H, 13.41%); δ 2.30 (t, *J* 7.5); ν/cm^{-1} 1735s (ester CO), 1200s (COC) and 1286m (C$H_2$$CO_2$); δ_H 2.29 (t, *J* 7.5) and 4.05 (t, *J* 6.7).

[1-$^3H_1$]Hexadecanal

Unlabelled hexadecanal (24 mg) was reduced with sodium borotritide (~ 2 mg, Amersham) in methanol (0.5 ml) by stirring at 25 °C overnight. Sodium borohydride (4 mg) was added to the mixture which was then stirred for 3 h to complete the reduction. Excess of borohydride was decomposed with 1 M hydrochloric acid (0.1 ml) and water (1 ml) and the mixture stirred with CH_2Cl_2 (8 ml) and $MgSO_4$ until clear. The extract was evaporated and the residue was dissolved in CH_2Cl_2 (1 ml), and stirred with CrO_3·pyridine complex (54 mg) for 18 h. Ether (8 ml) was added to the mixture which was then filtered through Celite and evaporated. The residue in CH_2Cl_2 was passed through a Silica gel column (Merck; 2 g) and eluted with CH_2Cl_2. The fractions which contained only hexadecanal (TLC) were collected and evaporated. The residue (~ 20 mg) was recrystallised with pure cold hexadecanal (280 mg) from 10 parts of methanol to give [1-$^3H_1$]hexadecanal (16 000 dpm/mg^{-1}).

[(1*R*)-$^2H_1$]Hexadecanol

The procedure of Midland *et al.*[17] and Brown and Pai,[18] was adopted. *S*-Alpine borane (0.5 M solution in tetrahydrofuran, Aldrich; 60 ml, 30 mmol) was added to a solution of [1-$^2H_1$]hexadecanal (2.41 g, 10 mmol) in tetrahydrofuran (50 ml) at 25 °C under dry N_2. The mixture was stirred for 46 h and cooled in an ice-bath. Acetaldehyde (2 ml) was added to the cooled mixture which was then stirred at 25 °C for 1 h to decompose excess of Alpine borane and finally evaporated *in vacuo*. The residue was dissolved in ether (300 ml) to which ethanolamine (2 ml) was added with stirring at 25 °C; stirring was then continued for 1 h. Insoluble material was filtered off and the filtrate was washed with 1M hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine and dried ($MgSO_4$). Evaporation *in vacuo* then gave an oily residue which was extracted with hexane. The extract was evaporated *in vacuo* and the residue was applied onto Silica Gel 60 (250 g, Merck), first washed with CH_2Cl_2 to remove non-polar material and then eluted with 2% methanol in CH_2Cl_2 to give [(1*R*)-$^2H_1$]hexadecanol (1.88 g, 77.4%, from hexane) mp 38–41 °C (Found: C, 78.71; H + D, 14.46. $C_{16}H_{33}DO$ requires C, 78.94; H + D, 14.49%); TLC R_F as for [1,1-$^2H_2$]hexadecanol above; $[\alpha]_{365}^{25}$ −2.10, $[\alpha]_{435}^{25}$ −1.16, $[\alpha]_{546}^{25}$ −0.68 and $[\alpha]_{578}^{25}$ −0.58 (*c* 25, $CHCl_3$); the triplet at δ 3.62 ppm (1-CH_2) in the ^{1}H NMR

spectrum of the unlabelled hexadecanol was present in this alcohol but it integrated for one proton; *m/z* (rel. intensity) 225.3 ($M - H_2O^{\bullet+}$, 1.0) and 224.3 ($M - 1 - H_2O^{\bullet+}$, 0.01). [(1*R*)-$^2H_1$]Hexadecyl tosylate had mp 47–48 °C after recrystallisation from methanol (Found: C, 69.28; H + D, 10.67; S, 8.31. $C_{23}H_{39}DO_3S$ requires C, 69.47; H + D, 10.39; S, 8.06%); R_F as above and the triplet at δ 4.00 ppm (1-CH_2) integrates for one proton). Similar reduction of [1-$^3H_1$]hexadecanal (113 mg) and conversion of the resulting alcohol as above gave [(1*R*)-$^3H_1$]hexadecyl tosylate (77 mg, 400 × 10^3 dpm).

[(1*S*)-$^2H_1$]Hexadecanol

This compound was similarly prepared from *R*-Alpine borane in 78% yield and had mp 37–40 °C (Found: 78.49; H + D, 14.54), R_F and ^{1}H NMR as for the *R*-enantiomer, $[\alpha]_{365}^{25}$ + 2.19, $[\alpha]_{435}^{25}$ + 1.26, $[\alpha]_{546}^{25}$ + 0.70, $[\alpha]_{578}^{25}$ + 0.63 (*c* 15, $CHCl_3$); *m/z* (rel. intensity) 225.3 ($M - H_2O^{\bullet+}$, 1.0) and 224.3 ($M - 1 - H_2O^{\bullet+}$, 0.015). [(1*S*)-$^2H_1$]Hexadecyl tosylate, mp 48–49 °C (Found: C, 69.33; H + D, 10.01; S, 8.28), [1,1-$^2H_2$]hexadecyl tosylate, mp 48–48.5 °C and [(1*S*)-$^3H_1$]hexadecyl tosylate (589 mg, 263 × 10^3 dpm) were prepared as described above.

[(1*R*)-$^2H_1$]Hexadecyl (1′*S*)-camphanate

This compound was prepared in 88% yield by mixing (−)-(1*S*)-camphanic acid chloride (Fluka; 217 mg, 1 mmol), [(1*R*)-^{2}H]hexadecan-1-ol (102 mg, 0.5 mmol) and dry pyridine (0.2 ml) in CH_2Cl_2 (1 ml) and stirring the mixture for 3 h at 25 °C. After this water (1 ml) was added to the mixture which was then stirred at 25 °C for 30 min and finally extracted with ether. The extract was washed with 1M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried (Na_2SO_4) and evaporated. The residue was recrystallised from methanol and had mp 53.5–55 °C (Found: C, 73.56; H + D, 11.46. $C_{26}H_{45}DO_4$ requires C, 73.71; H + D, 11.18%), $[\alpha]_{546}^{25}$ −10.1 (*c* 4, CCl_4); δ_H [in C_6D_6, δ 7.15, +1.2 mol of Eu(dpm)$_3$: and irradiation at *ca.* 1.4–1.6 ppm] 4.09 (s) assigned to 1′-H (equivalent to *pro*-H_S) by mixing with the [(1′*S*)-$^2H_1$] and unlabelled camphanate in various amounts. [(1*S*)-$^2H_1$]Hexadecyl (1′*S*)-camphanate was similarly prepared and had mp 54.5–55.5 °C (82% yield) (Found: C, 74.18; H + D, 11.00), $[\alpha]_{546}^{25}$ −10.9 (*c* 4, CCl_4); δ_H [in C_6D_6, δ 7.15, +1.2 mol of Eu(dpm)$_3$: and irradiation at *ca.* 1.4–1.6 ppm], 4.06 (s) and assigned to 1′-H (equivalent to *pro*-H_R) as with the previous isomer; and unlabelled hexadecyl (1′*S*)-camphanate (93%), had mp 55–55.5 °C (Found: C, 73.46; H, 11.26. $C_{26}H_{46}O_4$ requires C, 73.89; H, 10.97%), $[\alpha]_{546}^{25}$ −10.4 (*c* 3.7, CCl_4), δ_H [in C_6D_6, δ 7.15, +1.2 mol of Eu(dpm)$_3$: and irradiation at *ca.* 1.4–1.6 ppm] 4.11 (d, *J* 10.8, *pro*-H_R) and 4.08 (d, *J* 10.8, *pro*-H_S) ppm and were similarly prepared. All these esters had ν/cm^{-1} 1792 and 1731 (ester C=O) and 1171 (COC) and TLC: R_F (A) 0.00 (a) and 0.62 (b), (B) 0.00 (a) and 0.65 (b).

(1′*R*)-3-[1′-$^2H_1$]Hexadecyloxypropane-1,2-diol

Under a dry nitrogen atmosphere, sodium hydride (60% dispersed in oil; 24 mg, 0.5 mmol) was washed with pentane (discard washings), suspended in dry dimethylformamide (0.4 ml) and treated with (*RS*)-isopropylideneglycerol (53 mg, 0.4 mmol) in dimethylformamide (0.4 ml); the mixture was stirred at 25 °C until the sodium salt separated (~1 h, or 10 min at 80 °C). [(1*S*)-$^2H_1$]Hexadecyl tosylate (59 mg, 0.15 mmol) in dimethylformamide (0.4 ml) was added to the mixture which was then stirred at 80 °C until all the tosylate had reacted (1–4 h, by TLC). Ice-cold water (10 ml) was added with stirring to the mixture which was then extracted with ether. The extract was washed with saturated aqueous sodium hydrogen carbonate and brine, dried (Na_2SO_4), filtered and evaporated. The residue in pentane was passed through an alumina column (Brockman I, Merck; 5 g) and eluted with pentane. The fractions that contained the isopropylidene derivative were collected, evaporated and the residual oil was dissolved in MeOH (2 ml). One drop of concentrated hydrochloric acid was added to the solution and the mixture was set aside overnight. Crystals of the (1′*R*)-propanediol were collected and recrystallised by suspension in boiling pentane to which ether was added dropwise until all the solid dissolved; cooling of the mixture gave the product (50–80%), mp 64–65 °C (Found: C, 71.80; H + D, 12.86. $C_{19}H_{39}DO_3$ requires C, 71.87; H + D, 13.01%), $[\alpha]_{310}^{25}$ +2.4, $[\alpha]_{350}^{25}$ +1.69, $[\alpha]_{450}^{25}$ +1.00, $[\alpha]_{550}^{25}$ +0.45 (*c* 3.5, CH_2Cl_2); the ^{1}H NMR spectrum was similar to the spectra reported for chimyl alcohol[2,6] except that the triplet of doublets for the 1′,1′-protons at δ ~3.43 integrated for one proton instead of two; *m/z* (rel. intensity) 286.3 ($M - CH_3O^{\bullet+}$, 1.0) and 285.3 ($M - 1 - CH_3O^{\bullet+}$). (1′*S*)-3-[1′-$^2H_1$]Hexadecyloxypropane-1,2-diol (from [(1*R*)-$^2H_1$]hexadecyl tosylate), mp 63.5–64.5 °C (Found: C, 72.03; H + D, 12.88), $[\alpha]_{313}^{25}$ −1.84, $[\alpha]_{365}^{25}$ −0.89, $[\alpha]_{434}^{25}$ 0.50, $[\alpha]_{548}^{25}$ −0.22 (*c* 1.8, CH_2Cl_2); an ^{1}H NMR spectrum identical with that of the *R*-isomer; *m/z* (rel. intensity) 286.3 ($M - CH_3O^{\bullet+}$, 1.0) and 285.3 ($M - 1 - CH_3O^{\bullet+}$, 0.08). 3-[1′,1′-$^2H_2$]hexadecyloxypropane-1,2-diol (from 1,1-[$^2H_2$]hexadecyl tosylate), mp 62–63 °C (Found: C, 71.96; H + D, 13.57. $C_{19}H_{38}D_2O_3$ requires C, 71.65; H + D, 13.33%) with an ^{1}H NMR spectrum similar to those of the previous diols except that the multiplet at 3.40 was absent; (1′*R*)-3-[1′-$^3H_1$]hexadecyloxypropane-1,2-diol (10 mg, 74.3 × 10^3 dpm, from [(1*S*-$^3H_1$]hexadecyl tosylate) and (1′*S*)-3-[1′-$^3H_1$]hexadecyloxypropane-1,2-diol {41 mg, 300 × 10^3 dpm, from [(1*R*)-$^3H_1$]hexadecyl tosylate}, were all similarly prepared and had TLC: R_F (A) 0.00 (a), 0.62 (b), and (B) 0.00 and 0.65 (b).

(1′*R*)- and (1′*S*)-3-[1′-$^2H_1$]Hexadecyloxypropane-1,2-diol

These were also prepared under a nitrogen atmosphere by allowing isopropylideneglyceryl tosylate [0.57 g, 3 mmol; mp 49–49.5 °C (Found: C, 54.67; H, 6.39; S, 11.36. $C_{13}H_{18}O_5S$ requires C, 54.53; H, 6.34; S, 11.20%), prepared from isopropylidene glycerol and tosyl chloride in pyridine] in dimethylformamide (0.4 ml) to react with sodium [(1*R*)-$^2H_1$]hexadecylate and sodium [(1*S*)-$^2H_1$]hexadecylate, respectively [prepared from the alcohol (0.49 g, 2 mmol) and sodium hydride (0.072 g, 3 mmol, from a 60% dispersion in oil as above)] in dimethylformamide (0.8 ml); the mixture was heated and stirred at 100 °C overnight. Work-up as above gave, respectively, (1′*R*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diol, mp 63.5–64.5 °C (Found: C, 71.51; H + D, 13.17) and (1′*S*)-3-[1′-$^2H_1$]hexadecyloxypropane-1,2-diol, mp 63–64 °C (Found: C, 71.56; H + D, 13.20) in ~30% yields. The TLC, ^{1}H NMR spectrum and optical properties were the same as those of the respective propanediols above.

Enzyme kinetic measurements

The kinetic parameters of glyceryl-ether monooxygenase were determined with unlabelled, 3-[(1′*R*)-$^2H_1$]-, 3-[(1′*S*)-$^2H_1$]- and 3-[1′,1′-$^2H_2$]-hexadecyloxypropane-1,2-diol and (*RS*)-6-methyl-5,6,7,8-tetrahydropterin (100 μM) in 0.1 M Tris-HCl buffer (pH 7.4) and 25 °C using the enzyme preparation and assay procedures exactly as described previously.[2,6] The K_m and V_{max} values were evaluated from the kinetic runs using a computer program,[23] and are given in the discussion above.

Tritium released from the chiral propanediols during the enzymic reaction was determined as follows: a mixture of 1 M Tris-HCl buffer pH 7.4 (3.0 ml), water (16.8 ml), 1 mM hexadecyloxypropanediol in 0.8% of Mega-10 (3.0 ml), NADH (42.5 mg, 60 μM), catalase (3.0 ml, 3 mg, Sigma), human brain dihydropteridine reductase (0.2 ml, 26 μg protein),[24] and a microsomal preparation of glyceryl-ether monooxygenase from rat liver (2.5 ml, 50 mg of protein; specific activity of 27.7 nmol of batyl alcohol oxidised/min; mg protein, *cf.* ref 2) was incubated at 25 °C for 2 min, after which a 10 mM solution of (*RS*)-6-methyl-5,6,7,8-tetrahydropterin in 4 mM HCl (1.5 ml) was added to the mixture giving a total volume of 30 ml; the mixture was then kept at 25 °C. Aliquots (4 ml) were withdrawn at time intervals, placed in Quickfit test tubes containing formic

acid (0.4 ml) to quench the reaction. Each tube was connected to a longer tube *via* an inverted V bend with a tap at the apex. The apparatus was evacuated until completely degassed, the tap was closed and the longer tube was immersed into liquid nitrogen while the tube containing the frozen aliquot was at room temperature. All the water sublimed into the longer tube which was thawed and of which 3 ml were mixed with Aquasol (NEN Research Products; 3 ml); the radioactivity was then counted in polyethylene tubes in a Packard Tri-carb 300 scintillation counter. The aliquots (calculated on 4 ml) of (1′*R*)-3-[$^{3}H_{1}$]hexadecyloxypropane-1,2-diol had dpm (min); 16 (0.7), 31 (1.0), 22 (2.0), 26 (4.0), 33 (8.0), 53 (12) and 52 (20), whereas 3-[(1′*S*)-$^{3}H_{1}$]hexadecyloxypropane-1,2-diol had dpm (min): 25 (0.7), 45 (1.0), 74 (2.0), 135 (4.0), 222 (8.0), 260 (12.0) and 292 (20). The data show that after 20 min the *R*-isomer released 6.5% of the radioactivity, whereas the *S*-isomer released 37% of the radioactivity.

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