

Acid-catalyzed and Enzymatic Hydrolysis of *trans*- and *cis*-2-Methyl-3,4-epoxytetrahydropyran

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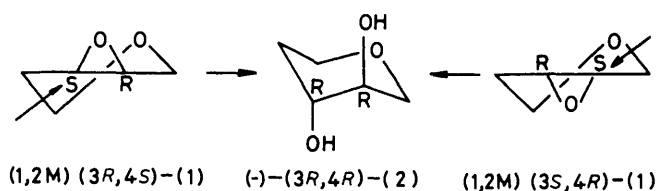
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Whereas the acid-catalyzed hydrolysis of *trans*- and *cis*-3,4-epoxy-2-methyltetrahydropyran gives the corresponding diols by opening both at C-4 and C-3 (64% attack at C-4 in the *trans*-epoxide, 86% in the *cis*-epoxide), the hydrolysis of the same substrates catalyzed by rabbit liver microsomal epoxide hydrolase is entirely regiospecific and involves in both cases exclusive attack at C-4. The racemic *cis*-epoxide reacts at a faster rate than the *trans*. The *2R,3R,4S*-enantiomer of the latter epoxide reacts at a much faster rate than its antipode to yield the (-)-(2*R*,3*R*,4*R*)-diol which is isolated at least 98% optically pure up to almost 50% conversion when starting from the racemic substrate. A reference sample of optically pure (+)-(2*S*,3*S*,4*S*)-diol was prepared from L-rhamnose. The enantiomeric excess of the (-)-(2*R*,3*R*,4*R*)-diol was also determined more precisely through g.l.c. analysis of diastereoisomeric MTPA esters. The present results confirm previous hypotheses on the topology of the hydrolase active site and emphasize the overriding importance of the orientation of a lipophilic substituent near the oxirane ring.

The epoxidation/hydrolysis sequence is of great importance in the metabolism of alkenes and arenes.¹ The two enzymes involved in these transformations, cytochrome P-450-dependent mono-oxygenase and epoxide hydrolase exhibit a relatively low substrate specificity, but the hydrolase (E.C.3.3.2.3) has a marked ability to discriminate between the diastereoisomers and enantiomers of particular substrates.²⁻⁹

Recent work from this laboratory^{7,9-11} concerning the enzymatic hydrolysis of epoxide rings fused to six-membered rings has provided evidence for the topology of the enzyme active site, the main features being that (i) chair-like transition states deriving from antiparallel attack by water on epoxy-cyclohexanes and epoxytetrahydropyrans having the six-membered ring in a monoplanar conformation of 3,4 M helicity appear to be preferentially stabilized by the enzyme; (ii) a higher degree of stabilization is provided by the presence of lipophilic substituents placed to the right of the epoxy oxygen if the substrate in the active site of the enzyme is viewed with the oxygen on the top side, thus indicating the presence of a lipophilic pocket similarly oriented in the active site. A particularly intriguing result was obtained with (±)-3,4-epoxytetrahydropyran (1)¹¹ in which both enantiomers on enzymatic hydrolysis formed the same diol, (-)-(3*R*,4*R*)-*trans*-tetrahydropyran-3,4-diol (2) at practically equal rates, nucleophilic attack by water taking place exclusively at position 3 of the (3*S*,4*R*) enantiomer and at position 4 of the (3*R*,4*S*) enantiomer (Scheme 1). This proved that the high preference for a diaxial opening of the 1,2 M conformations overwhelms the preference for nucleophilic attack at C-4 observed in acid-catalyzed, and to a lesser extent in base-catalyzed epoxide ring-opening reactions.

We therefore thought it interesting to extend our study to the hydrolysis of alkyl substituted derivatives of (1), *trans*- and *cis*-2-methyl-3,4-epoxytetrahydropyran (8) and (10), which can both exist in two half-chair conformations of different stabilities. The methyl group vicinal to the epoxide ring could on the one hand enhance the reactivity of one of the enantiomers because of favourable hydrophobic interactions in the active site, and on the other hand decrease it because of steric hindrance to approach of the water molecule. We also wanted to compare the composition of diols obtained in the enzymatic reactions with that of the diols formed in an acid-catalyzed hydrolysis.

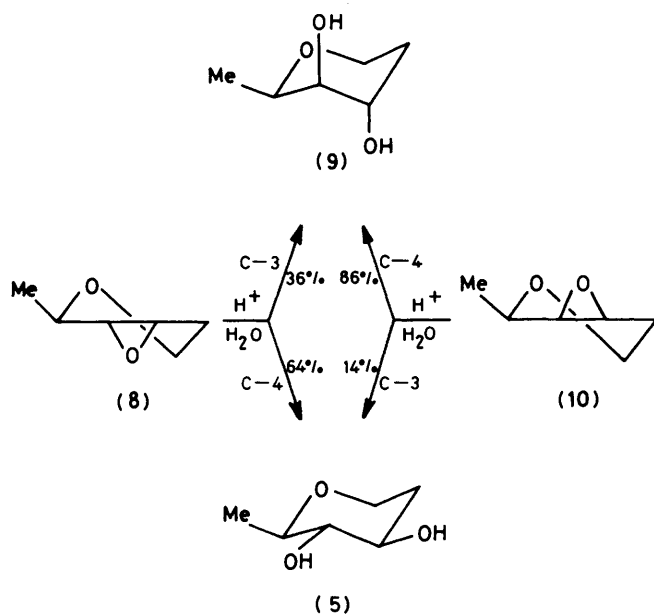


Scheme 1.

Results

The epoxides (8) and (10) have been prepared previously.¹² The corresponding racemic diols (5) and (9) were prepared for comparison with the enzymatic hydrolysis products, by subjecting both (±)-(8) and a mixture of (±)-(8) and (±)-(10) to a treatment with dilute aqueous sulphuric acid. It was thus established that both (8) and (10) give mixtures of the diols (5) and (9) in a ratio of 64 : 36 from (8) and 14 : 86 from (10). The diols were separated by chromatography on alumina. The structure of (±)-(9) was confirmed by the n.m.r. spectrum of its di-(*p*-nitrobenzoate). The half-band widths of the signals for the protons α to the benzyloxy groups show that these groups are axial and the methyl group equatorial. The diol (±)-(5) was identified through an independent synthesis of the (+)-enantiomer from L-rhamnose (3) involving the sequence shown in Scheme 2, *via* L-diacetylramnal (4), which was hydrogenated to give (+)-(2*S*,3*S*,4*S*)-(5), $[\alpha]_D^{25} +2.8^\circ$, having i.r. and n.m.r. spectra identical with those of (±)-(5).

The epoxide hydrolase catalyzed hydrolyses of the racemic compounds (8) and (10) were then investigated. All the incubations were run with microsomes having the same activity and in a range of substrate concentrations (0.017–0.090M) certainly far in excess of the K_m of the enzyme,⁷ in order to ensure a meaningful comparison of the result obtained from different substrates and different times of reaction. Percentages of conversion were determined by direct injection of the incubation solution into the g.l.c. column (see Experimental section). Preliminary experiments showed that only the diequatorial diol (5) and the diaxial diastereoisomer (9) were formed as the enzymatic hydrolysis products of (±)-(8) and (±)-(10) respectively (g.l.c. analysis would have revealed the presence of <1% of the diequatorial diol in a sample of the

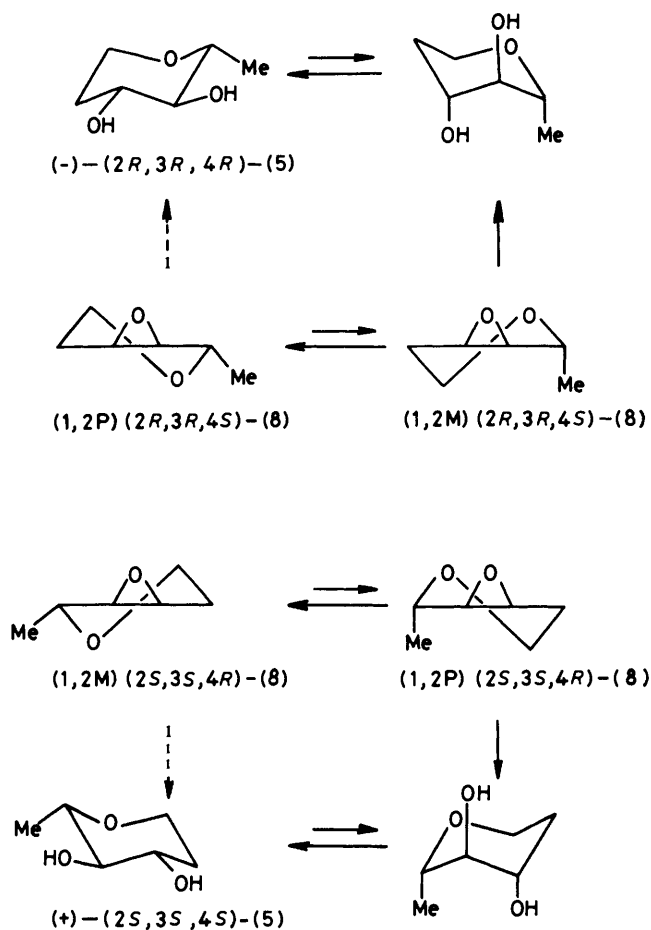


Scheme 3.

ing inductive effect of the tetrahydropyran ring oxygen, which is stronger on the nearer C-O bond. Also in the present case, the regioselectivity is higher in the *cis*-epoxide (10) because, as shown in Scheme 3, attack on C-4 in this epoxide can occur in the diaxial fashion; on the more stable conformation with an equatorial methyl group. In contrast, in the *trans*-epoxide (8) attack on C-4 must either involve diequatorial opening on the more stable conformation, or diaxial opening on the less stable one with an axial methyl group (not shown in Scheme 3).

The hydrolase-catalyzed hydrolysis of (8) and (10), is, on the other hand, entirely regiospecific, the *trans*-epoxide giving exclusively the diequatorial diol (9), and the *cis*-epoxide the diaxial diol (9): attack by water always takes place exclusively at C-4. This result is shown to be surprising by comparison with those results previously obtained with the unsubstituted 3,4-epoxycyclohexane (Scheme 1). In the latter, attack by water took place at almost the same rate on C-3 in one enantiomer and on C-4 in the other; this showed that, in contrast with the acid-catalyzed hydrolysis, the inductive effect of the tetrahydropyran ring oxygen plays little, if any role in the enzymatic hydrolysis. This behaviour indicates that the tetrahydropyran ring appears to differ little from a cyclohexane ring in such situations. Thus, the fitting of the substrate in the enzyme active site and steric factors around the oxirane ring rather appear to be the main factors in determining the regiospecificity of attack by water.

The results obtained with (8) and (10) can be roughly compared with those previously observed⁹ with the 3-*t*-butyl-1,2-epoxycyclohexanes. Although the latter are much poorer substrates, probably because of the bulk of the substituent, the *cis*-isomer is hydrolysed much more rapidly than the *trans*-isomer; the 1*S*,2*R*,3*R*-enantiomer of the *trans*-epoxide is slowly hydrolysed, whereas the 1*R*,2*S*,3*S*-enantiomer is entirely resistant to enzymatic hydrolysis. Although we have yet to study the hydrolase-promoted hydrolysis of the *cis*-epoxide (10) in detail, it appears from preliminary data (see Figure) that one of its enantiomers is hydrolysed more rapidly than the other. The more detailed analysis of the *trans*-epoxide (8) hydrolysis has indicated that the 2*R*,3*R*,4*S*-enantiomer, corresponding to the 1*S*,2*R*,3*R*-configuration of *trans*-3-*t*-



Scheme 4.

butyl-1,2-epoxycyclohexane, is hydrolysed much more rapidly than the 2*S*,3*S*,4*R*-enantiomer, the difference in rates being sufficient to ensure isolation of almost optically pure 2*R*,3*R*,4*R*-diol up to almost 50% reaction (see Scheme 4). After all this enantiomer of the epoxide is used up, the enzymatic reaction proceeds much more slowly and practically stops at *ca.* 70% conversion. The fact that a sharp decrease, instead of an increase in rate after 50% conversion was observed, rules out competitive inhibition by the slower reacting enantiomer on the hydrolysis of the faster reacting one, as was found to operate with the enantiomers of styrene oxide.¹³ Rather it appears that a better fit of the 2*R*,3*R*,4*S*-enantiomer into the enzyme active site is responsible for the observed behaviour, as was hypothesized for the *trans*-3-*t*-butyl-1,2-epoxycyclohexanes. Bearing in mind the hypothesis mentioned at the beginning on the factors affecting the enantioselectivity of these reactions, the higher reactivity of the 2*R*,3*R*,4*S*-enantiomer is in agreement with the assumption that reactivity is enhanced by the presence of a lipophilic substituent at the right of the epoxy oxygen, if the substrate in the active site is viewed with the oxirane ring on the topside as shown in Scheme 4.

The fact that (8) is a much better substrate than the unsubstituted 3,4-epoxycyclohexane (1), is in accord with a favourable hydrophobic interaction between the lipophilic alkyl group and the active site. The second hypothesis, that 1,2 *M* helicity of the six-membered ring facilitates the hydrolytic process, coupled with the well-known Fürst-Plattner rule about the preferential diaxial opening of epoxide rings, makes

it very probable that the epoxide reacts in its less stable conformation with an axial methyl group. The advantage of diaxial opening in a favourable conformation would certainly offset the disadvantage of the higher-energy conformation in the transition state. On the other hand, the wrongly placed methyl group and the diequatorial ring opening in the 1,2 M conformation of the 2*S*,3*S*,4*R*-enantiomer accounts for its much slower rate of hydrolysis.

The fact that (2*R*,3*R*,4*S*)-(8) is hydrolysed at a much higher rate than the configurationally equivalent (1*S*,2*R*,3*R*)-*trans*-3-*t*-butyl-1,2-epoxycyclohexane, can well be explained by the conformation rigidity of the latter compound that obliges it to react by diequatorial opening in an unfavourable 3,4 P conformation.

Finally, the complete and exclusively regiospecific attack by water at C-4 in the enzymatic reaction, contrasts with the results of the acid catalyzed hydrolysis, and seems to point to an important steric effect of the methyl group which opposes attack at the vicinal oxirane carbon.

Experimental

M.p.s (Kofler block) are uncorrected; i.r. spectra, taken on neat liquids or on paraffin oil mulls of solids on a Perkin-Elmer 197, were used for comparison between compounds; n.m.r. spectra were determined on *ca.* 10% solutions in CDCl₃ on a Varian T60 and on a Varian CFT20 spectrometer with SiMe₄ as internal standard. Optical rotations were measured in the indicated solvents with a Perkin-Elmer 141 photo-electric polarimeter. The mass spectra were determined on a Varian CH7 spectrometer with 70 eV ionization energy and a 300 μ A trap current; g.l.c. analyses were carried out with a Dani 3800 instrument under the following conditions: for (5), (8), (9), and (10), a glass column (2.5 mm i.d. \times 1.5 m) packed with 10% Carbowax 20M on silanized Chromosorb W, 80–100 mesh, low isotherm 120 °C (0 min), high isotherm 200 °C, temperature increment 8 °C/min, N₂ flow 30 ml/min, relative retention times of (5), (8), (9) and (10), 5.41, 1.00, 6.51, 1.46, for the MTPA derivatives (6) and (7), capillary glass column (0.25 mm i.d. \times 20 m) packed with SE52, column temperature 200 °C, N₂ flow 1.5 ml/min, relative retention times of (6) and (7), 1.00 : 1.03. Preparative t.l.c. was carried out on 20 \times 20 cm silica gel plates, 2 mm thick (PSC Fertigtplatten Kieselgel 60 F₂₅₄ Merck). Light petroleum was the fraction of boiling range 40–60 °C.

trans- and *cis*-2-Methyl-3,4-epoxytetrahydropyran (8) and (10).—These compounds were obtained as previously reported.¹² L-Rhamnose (Fluka) was used without purification. Optically pure MTPA chloride was prepared from commercially available (–)- α -methoxy- α -trifluoromethylphenylacetic acid (EGA CHEMIE) according to the reported procedure.¹⁴

(\pm)-*t*-2- and (\pm)-*c*-2-Methyltetrahydropyran-*r*-3,*t*-4-diol (5) and (9).—A 50 : 50 mixture of *trans*- and *cis*-2-methyl-3,4-epoxytetrahydropyran (8) and (10) (1.5 g) was dissolved in 0.5M-sulphuric acid (15 ml) and stirred for 4 h at 40–50 °C; the neutralized (solid NaHCO₃) solution was evaporated to dryness under reduced pressure, and the residue extracted with hot acetone (3 \times 50 ml) and evaporated to give in almost quantitative yield a mixture of the racemic diols (5) and (9) in the ratio of 39 : 61 (g.l.c.) (1.7 g), b.p. 94–106 °C at 0.4 mmHg. An identical treatment of the pure *trans*-epoxide (8), gave a mixture of racemic (5) and (9) in the ratio of 64 : 36 (g.l.c.). Chromatography over basic alumina (activity I, 2 cm i.d. \times 30 cm column) of the 39 : 61 mixture of racemic (5) and (9) with dichloromethane–methanol (95 : 5) as the eluant allowed

complete separation of (\pm)-(9) (eluted first) and (\pm)-(5). Crystallization from dichloromethane–light petroleum gave (\pm)-(5) as a white solid (needles), m.p. 102–104 °C (Found: C, 54.75; H, 8.9. C₆H₁₂O₃ requires C, 54.53; H, 9.15%), δ (CDCl₃) 1.33 (3 H, d, *J* 6 Hz, CH₃), 1.4–2.2 (2 H, m, 5-H₂), 2.8–4.1 (5 H, m, 2-, 3-, 4-H and 6-H₂) and 4.28 (2 H, s, 2 \times OH). Compound (\pm)-(9) was obtained as a viscous liquid by bulb-to-bulb distillation at 90–95 °C (bath temp.) (at 0.3 mmHg), δ (CDCl₃) 1.2 (3 H, s, *J* 7 Hz, CH₃), 1.5–2.4 (2 H, m, 5-H₂), 2.3 (2 H, s, 2 \times OH), 3.4 (1 H, m, *w*₄ 8 Hz), and 3.6–4.2 (4 H, m) (2-, 3-, 4-H, and 6-H₂). The di-(*p*-nitrobenzoate) of (\pm)-(9) was obtained by treatment of pure diol (9) (0.25 g) with benzoyl chloride (0.76 g) in dry pyridine (10 ml) for 5 h on a steam-bath; this was followed by treatment with cold 2M-hydrochloric acid and extraction with chloroform. The crude di-(*p*-nitrobenzoate) (0.3 g) was chromatographed on silica gel in chloroform and crystallized from dichloromethane–pentane (needles), m.p. 228–230 °C (Found: C, 55.9; H, 4.2; N, 6.35. C₂₀H₁₈N₂O₉ requires C, 55.82; H, 4.22; N, 6.51%); δ (CDCl₃) 1.26 (3 H, d, *J* 6.5 Hz, CH₃), 1.6–2.5 (2 H, m, 5-H₂), 3.7–4.4 (3 H, m, 2-H and 6-H₂), 5.18 (1 H, m, *w*₄ 7 Hz) and 5.46 (1 H, m, *w*₄ 6 Hz)(3- and 4-H), and 8.4 (10 H, m, ArH).

L-Diacetyl-rhamnal (4).—A mixture of α - and β -tetraacetyl-rhamnose obtained by acetylation of L-rhamnose monohydrate (3.0 g) with acetic anhydride (10 ml) in dry pyridine (20 ml) for 48 h at room temperature, was treated at 0 °C with a 40% solution of hydrobromic acid in glacial acetic acid (50 ml) and stirred until it was completely solubilized (90 min). After being stirred for an additional 1 h the mixture was diluted with dichloromethane (50 ml), thrice washed with ice-water (50 ml), dried (K₂CO₃), filtered, and evaporated to give the crude tri-*O*-acetyl-L-rhamnopyranosyl bromide as an amorphous solid (4.2 g), $[\alpha]_D^{20}$ –151° (*c* 1.0, CHCl₃) [lit.,¹⁵ $[\alpha]_D^{25}$ –172° (*c* 1.0, CHCl₃) for purified product]. A solution of this crude product in glacial acetic acid (20 ml) was added at –5 °C with stirring to a suspension in aqueous acetic acid (30 ml) of sodium acetate trihydrate (2.6 g) and powdered zinc (7.9 g) previously activated by addition of a solution of copper sulphate pentahydrate (0.7 g) in water (3 ml). After the mixture had been stirred at –5 °C for 2 h, excess of zinc was filtered off, and the solution was extracted with chloroform (100 ml); the organic layer was then separated, washed with sodium hydrogencarbonate, dried (MgSO₄), filtered, and evaporated to give (4) (1.1 g), b.p. 120 °C (Kugelrohr) (at 0.5 mmHg) [lit.,¹⁶ b.p. 68–69 °C (at 0.06 mmHg)], $[\alpha]_D^{21}$ +66.9° (*c* 0.9, CHCl₃) [lit.,¹⁷ $[\alpha]_D^{18}$ +68.0°, (CHCl₃)] δ (CDCl₃) 1.2 (3 H, d, *J* 6.5 Hz, CH₃), 2.02 (3 H, s) and 2.08 (3 H, s) (3- and 4-OCOCH₃), 3.95br (1 H, sextet, *J* 7 Hz, 5-H), 4.6–5.3 (3 H, m, 2-, 3-, and 4-H), and 6.4br (1 H, d, *J* 7 Hz, 1-H).

(+)-(2*S*,3*S*,4*S*)-*t*-2-Methyltetrahydropyran-*r*-3,*t*-4-diol (+)-(5).—A solution (0.85 g) of diacetyl-L-rhamnal (4) in 50% aqueous ethanol (20 ml) was stirred under a small overpressure of hydrogen (100 cm water) in the presence of Adams PtO₂ (0.01 g); the theoretical amount of hydrogen was rapidly adsorbed (1 h). The catalyst was filtered off and the solution evaporated under reduced pressure to give the crude diacetate which was directly solubilized in methanol (10 ml) and treated for 3 h under reflux with 3M-sodium hydroxide (10 ml). The solution was filtered through Amberlyst 15 resin to give, after evaporation of solvent, crude (+)-(5) (0.46 g, 88%). Chromatography over silica gel (1 cm \times 10 cm column) with dichloromethane–methanol (9 : 1) as eluant afforded liquid (+)-(5) with retention time, i.r. and n.m.r. spectra identical with an authentic sample of (\pm)-(5). Bulb-to-bulb

distillation at 100–105 °C (bath temp.) (at 0.5 mmHg) afforded an amorphous white solid (m.p. not determinable because of its high hygroscopicity) which had $[\alpha]_D^{25} +2.8^\circ$ (c 3.7, EtOH).

Microsomal Preparations.—Liver microsomes, prepared from phenobarbital-treated male New Zealand white rabbits as previously described,⁷ were suspended in 0.01M-Tris-hydrochloric acid buffer (pH 9.0) to a final protein concentration of ca. 15 mg/ml and stored at –40 °C.

Incubations.—Analytical experiments were carried out with the following standard procedure: a solution of 4 mg of epoxide in 100 μ l of acetonitrile was added to 2 ml of microsome suspension (15 mg/ml proteins) and the mixture was incubated by shaking at 37 °C. After the times reported in the Figure, the incubations were terminated by cooling and the percentages of conversion were determined on the basis of the ratios of diol to epoxide observed by direct injection of the incubation mixture into the g.l.c. column. G.l.c. analysis showed that only the diequatorial diol (5) was formed from (8) and the diaxial diol (9) from (10). Each value is the average of at least three determinations, corrected by using a calibration curve obtained with standard solutions of the pure reference compounds in 0.01M-Tris-hydrochloric acid buffer (pH 9.0). For preparative-scale incubations the epoxide (8) (100 mg) in acetonitrile (300 μ l) was added to microsome suspension (10 ml; 15 mg/ml proteins) and the mixture was incubated by shaking at 37 °C. After the times reported in the Table, the incubation mixtures were cooled and analysed by g.l.c. (see above). The diol (5) was obtained by extraction of the mixture with ethyl acetate (3 \times 20 ml) with vigorous shaking for 5 min. The extracts were dried (MgSO₄) and evaporated under reduced pressure. Blank experiments carried out with pure compounds (1), (8) and (10) and boiled microsomes and with pure racemic (2), (5), and (9) and active microsomes showed that no spontaneous hydrolysis of the epoxides occurred even at the longest incubation times and that the racemic diols were recovered from incubations in 70–85% yields.

Enantiomeric Composition of the Diol (5).—The enantiomeric excess (e.e.) of the diol (5) obtained in the enzyme-promoted hydrolysis of the epoxide (8) was determined by conversion into the diastereoisomeric MTPA esters (6) and (7) according to the following procedure. The diol (5) (0.03 g) dissolved in dry pyridine (5 ml) in the presence of 3 Å molecular sieves, was treated for 24 h at room temperature with (–)- α -methoxy- α -trifluoromethylphenylacetic acid chloride (0.15 g). The reaction mixture was hydrolysed with water (1 ml) and extracted with ether (20 ml); the extract was then washed with 10% aqueous hydrochloric acid (20 ml) and saturated aqueous sodium carbonate (20 ml). The solution was analysed by g.l.c. (capillary column): (±)-(5) gave a mixture of (6) and (7) in a 50 : 50 ratio. When a ratio of diol to acid

chloride of 1 : 1 was used, the ratio of (6) and (7) was 50 : 50 and ca. 60% of unchanged diol was recovered. An identical treatment on (+)-(5) derived from L-rhamnose gave pure (7). The products derived from the diols formed after 25, 40, and 60 min of incubation showed only the peak corresponding to (6) [$<1\%$ of diastereoisomer (7)]. A 50 : 50 mixture of (6) and (7) from (±)-(5) was purified by preparative t.l.c. on silica gel in dichloromethane–methanol (98 : 2) (Found: C, 55.35; H, 5.65. C₁₆H₁₉F₃O₅ requires C, 55.17; H, 5.50%); δ (CDCl₃) 1.30–1.32 (3 H, 2d, *J* 5.8 Hz, CH₃), 1.6–2.3 (2 H, m, 5-H₂), 3.1–4.0 (4 H, 3m, 2-, 3-H, and 6-H₂), 3.55–3.58 (3 H, 2s, OCH₃), 5.0 (1 H, m, *w*_{1/2} 25 Hz, 4-H), and 7.4 (5 H, m, C₆H₅); *m/z* 190 (14), 189 (100), 170 (35), 115 (61), and 105 (31).

Acknowledgements

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