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

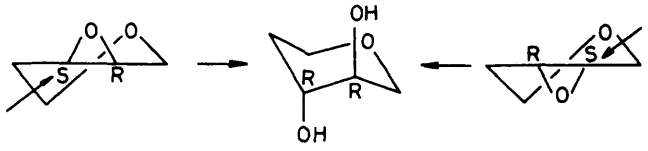
Giorgio Catelani and Ettore Mastrorilli *
Istituto di Chimica organica della Facoltà di Farmacia dell'Università di Pisa, Via Bonanno 6, 56100 Pisa, Italy

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 2*R*,3*R*,4*S*-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 epoxycyclohexanes 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 topside, 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.



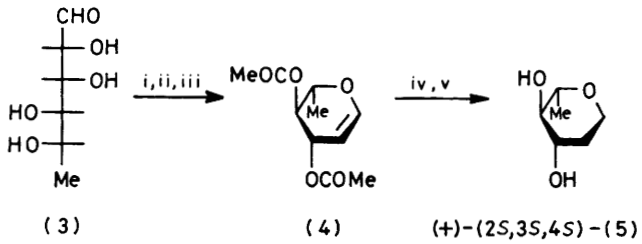
(1,2M) (3*R*,4*S*)-(1) (-)-(3*R*,4*R*)-(2) (1,2M) (3*S*,4*R*)-(1)

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 benzoyloxy 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-diacetylrhamnal (4), which was hydrogenated to give (+)-(2*S*,3*S*,4*S*)-(5), $[\alpha]_D^{25}$ +2.8°, 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



(3) (4) (+)-(2S,3S,4S)-(5)

Scheme 2. *Reagents:* i, Ac_2O, Py; ii, HBr, glacial CH_3CO_2H; iii, Zn–$CuSO_4$, AcOH–H_2O; iv, H_2–PtO_2, MeOH–H_2O; v, NaOH, MeOH

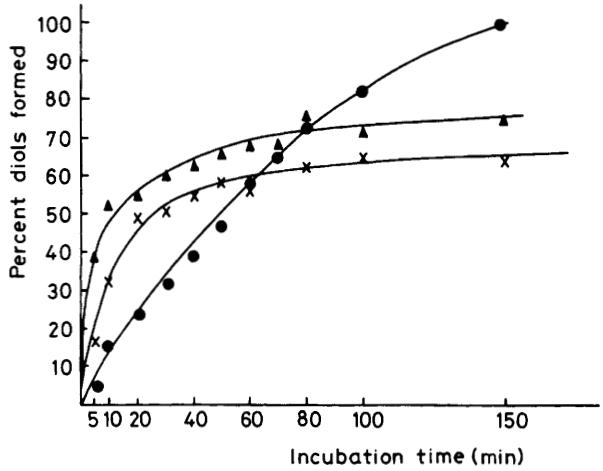


Figure. Enzymatic hydrolysis of (1), (8), and (10). The incubations were carried out at 37 °C. Substrate concentrations *ca.* 0.017M; protein concentrations 15 mg ml^{-1} (see Experimental section). (●) *trans*-Tetrahydropyran-3,4-diol (2); (×) *t*-2-methyltetrahydropyran-*r*-3,*t*-4-diol (5); (▲) *c*-2-methyltetrahydropyran-*r*-3,*t*-4-diol (6)

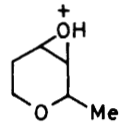
diaxial diol and *vice versa*). The results of the hydrolyses of (±)-(8) and (±)-(10) and of the unsubstituted 3,4-epoxytetrahydropyran are shown in the Figure in terms of diol formation against time.

The enzymatic hydrolysis of the racemic *trans*-epoxide (8) was examined in greater detail because of the availability of one of the enantiomeric diols. The diols from incubations carried out on larger amounts of substrate were extracted with ethyl acetate from the residue of the reaction solution after concentration to dryness under reduced pressure and subjected to measurement of optical rotations. The results obtained at different incubation times are given in the Table. Below 50% conversion, hydrolysis of (±)-(8) was fairly rapid. The rate was lowered considerably after 50% conversion and hydrolysis practically came to a halt after *ca.* 70% of the glycol had formed. The optical rotation of (5) at different percentages of enzymatic hydrolysis of racemic (8) remained practically unchanged until *ca.* 50% conversion; it was then almost equal to that of the optically pure diol obtained from L-rhamnose. However, the low specific rotation of (5) makes polarimetric measurements rather unprecise and subject to a high degree of error. It was, therefore, necessary to check the enantiomeric excess (e.e.) by converting the diol (5), obtained from the enzymatic hydrolysis, into diastereoisomeric (−)-*R*-(α-methoxy-α-trifluoromethylphenylacetic acid) (MTPA) esters (6) and (7). It was found that reaction with the acid chloride in pyridine at room temperature gave almost exclusively the mono esters even in the presence of an excess of MTPA chloride. This was evident from elemental analysis, from the n.m.r. spectrum (see Experimental section), and from the mass

Table. Conversion of (±)-*trans*-2-methyl-3,4-epoxytetrahydropyran (8) into *t*-2-methyltetrahydropyran-*r*-3,*t*-4-diol (5)

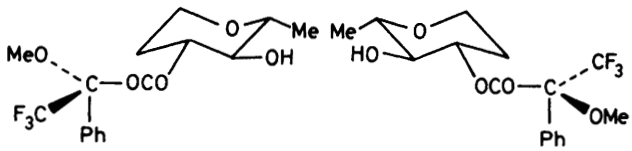
Incubation time (min)	% Hydrolysis	Diol (5) $[\alpha]_D^{25}$ (*c* 3.0, EtOH) (°)	E.e. %[a]
25	27	−2.0	98
40	40	−2.5	98
60	50	−2.0	98
150	60	−1.7	66

[a] Obtained from the ratios of diastereoisomeric MTPA esters (6) and (7).



A

spectrum which showed no peak for M^+, but did exhibit an abundant peak at *m/z* 189, possibly due to ion A.

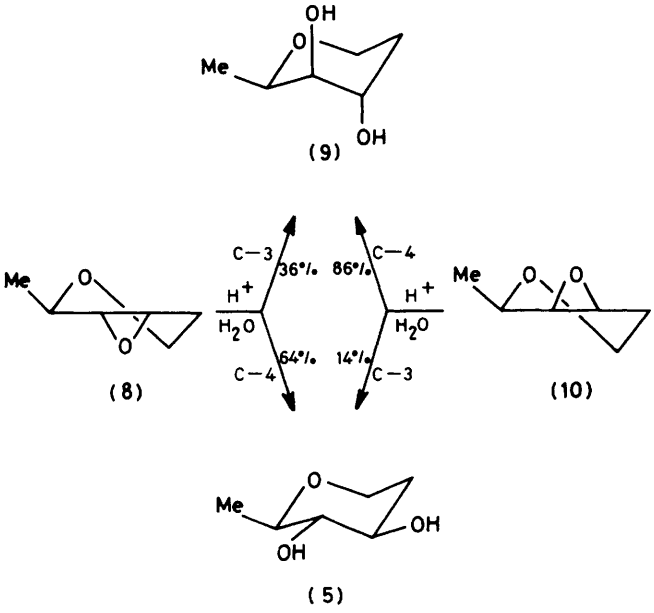


(6) (7)

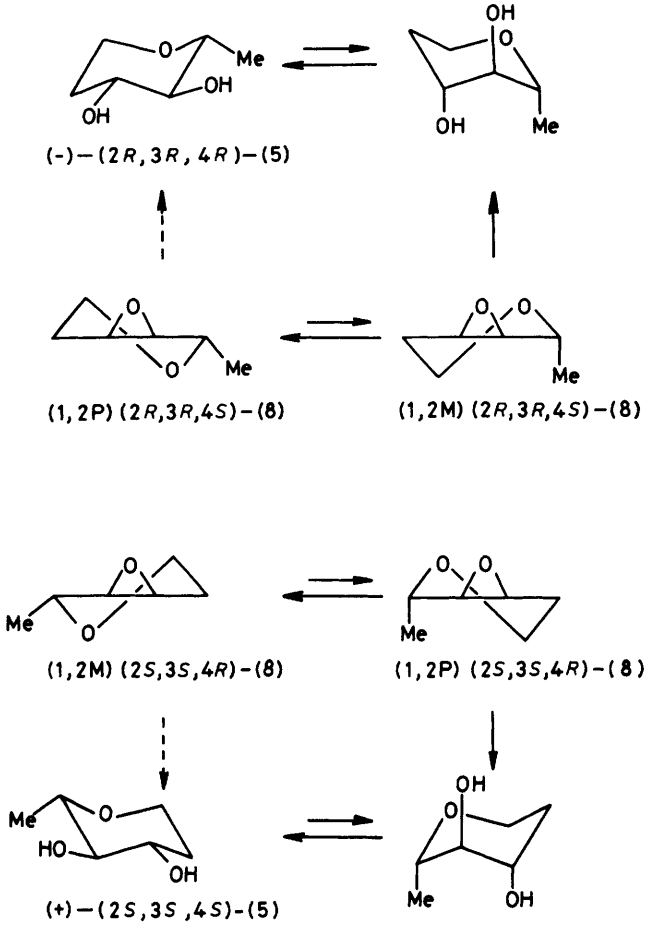
The hydroxy group which is esterified by MTPA chloride is most likely to be the one at position 4; it is less hindered than the one at position 3 and should react much more rapidly. The introduction of a second bulky acyl group would be severely hindered by the two vicinal substituents (methyl and methoxytrifluoromethylphenylacetyl), and would, therefore, be extremely slow under the mild acylation procedure. An effect of asymmetric induction in the case of incomplete esterification that might affect the values of e.e., is unimportant. This is evidenced by the fact that in a reaction of MTPA chloride with an excess of the racemic diol, in which a large part of the diol remained unchanged, g.l.c. analysis showed that the two diastereoisomers (6) and (7) were present in equal amounts. Analysis of the diastereoisomeric esters (6) and (7) by n.m.r. was not convenient since the methyl doublets were separated by only 2 Hz, the methoxy singlets by 2.5 Hz. They were however easily analysed by g.l.c. on a 20-m capillary column. The product from the racemic diol gave two separate peaks with the same area; that corresponding to the (+)-diol obtained from L-rhamnose was the peak with the longer retention time. Analysis of the product obtained in the enzymatic reaction confirmed the exclusive (>98% e.e.) presence of the (2*R*,3*R*,-4*R*)-diol in up to 50% conversion; at higher conversion the e.e. decreased to *ca.* 66% when the reaction stopped.

Discussion

The acid-catalyzed hydrolysis of the *trans*- and *cis*-epoxides (8) and (10) shows a significant preference for attack by water at C-4, as previously found [12] for the reactions of the same epoxides with other nucleophiles (HCl, HBr, $LiAlH_4$). This preference was attributed to an epoxide C–O bond-strengthen-



Scheme 3.



Scheme 4.

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 (5), 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-epoxytetrahydropyran (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 observed [9] 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-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 reponsible 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 lipophylic 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-epoxytetrahydropyran (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 $CDCl_3$ on a Varian T60 and on a Varian CFT20 spectrometer with $SiMe_4$ 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 μA 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. × 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, N_2 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. × 20 m) packed with SE52, column temperature 200 °C, N_2 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 × 20 cm silica gel plates, 2 mm thick (PSC Fertigplatten Kieselgel 60 F_{254} 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]

(±)-t-2- *and* (±)-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 $NaHCO_3$) solution was evaporated to dryness under reduced pressure, and the residue extracted with hot acetone (3 × 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. × 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 (±)-(9) (eluted first) and (±)-(5). Crystallization from dichloromethane–light petroleum gave (±)-(5) as a white solid (needles), m.p. 102—104 °C (Found: C, 54.75; H, 8.9. $C_6H_{12}O_3$ requires C, 54.53; H, 9.15%), δ ($CDCl_3$) 1.33 (3 H, d, *J* 6 Hz, CH_3), 1.4—2.2 (2 H, m, 5-H_2), 2.8—4.1 (5 H, m, 2-, 3-, 4-H and 6-H_2) and 4.28 (2 H, s, 2 × OH). Compound (±)-(9) was obtained as a viscous liquid by bulb-to-bulb distillation at 90—95 °C (bath temp.) (at 0.3 mmHg), δ ($CDCl_3$) 1.2 (3 H, s, *J* 7 Hz, CH_3), 1.5—2.4 (2 H, m, 5-H_2), 2.3 (2 H, s, 2 × OH), 3.4 (1 H, m, $w_{\frac{1}{2}}$ 8 Hz), and 3.6—4.2 (4 H, m) (2-, 3-, 4-H, and 6-H_2). The di-(*p*-nitrobenzoate) of (±)-(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. $C_{20}H_{18}N_2O_9$ requires C, 55.82; H, 4.22; N, 6.51%); δ ($CDCl_3$) 1.26 (3 H, d, *J* 6.5 Hz, CH_3), 1.6—2.5 (2 H, m, 5-H_2), 3.7—4.4 (3 H, m, 2-H and 6-H_2), 5.18 (1 H, m, $w_{\frac{1}{2}}$ 7 Hz) and 5.46 (1 H, m, $w_{\frac{1}{2}}$ 6 Hz)(3- and 4-H), and 8.4 (10 H, m, ArH).

L-*Diacetylrhamnal* (4).—A mixture of α- and β-tetra-acetylrhamnose 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 (K_2CO_3), 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, $CHCl_3$) {lit.,[15] $[\alpha]_D^{25}$ −172° (*c* 1.0, $CHCl_3$) 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 ($MgSO_4$), 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, $CHCl_3$) {lit.,[17] $[\alpha]_D^{18}$ +68.0°, ($CHCl_3$)} δ ($CDCl_3$) 1.2 (3 H, d, *J* 6.5 Hz, CH_3), 2.02 (3 H, s) and 2.08 (3 H, s) (3- and 4-$OCOCH_3$), 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).

(+)-(2S,3S,4S)-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 PtO_2 (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 × 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 (±)-(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° (*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 μl 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 μl) 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 × 20 ml) with vigorous shaking for 5 min. The extracts were dried ($MgSO_4$) 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. $C_{16}H_{19}F_3O_5$ requires C, 55.17; H, 5.50%); δ ($CDCl_3$) 1.30—1.32 (3 H, 2d, *J* 5.8 Hz, CH_3), 1.6—2.3 (2 H, m, 5-H_2), 3.1—4.0 (4 H, 3m, 2-, 3-H, and 6-H_2), 3.55—3.58 (3 H, 2s, OCH_3), 5.0 (1 H, m, $w_{\frac{1}{2}}$ 25 Hz, 4-H), and 7.4 (5 H, m, C_6H_5); *m/z* 190 (14), 189 (100), 170 (35), 115 (61), and 105 (31).

Acknowledgements

This work was supported by grants from Ministero della Pubblica Istruzione and Consiglio Nazionale delle Ricerche. We thank Professor Giancarlo Berti for fruitful discussions of this work.

References

1 F. Oesch, *Xenobiotica*, 1973, **3**, 305; T. M. Guenthner and F. Oesch, ' Polycyclic Hydrocarbons and Cancer ', eds H. V. Gelboin and Ts'O P. On Pong, Academic Press, New York, 1981, vol. 3, p. 183.
2 D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltmann-Niremberg, and S. Underfriend, *J. Am. Chem. Soc.*, 1968, **90**, 6525.
3 D. M. Jerina, M. Ziffer, and J. W. Daly, *J. Am. Chem. Soc.*, 1970, **92**, 1056.
4 D. R. Takker, H. Yagi, W. Levin, A. Y. H. Lu, A. H. Conney, and D. M. Jerina, *J. Biol. Chem.*, 1977, **252**, 6328.
5 R. P. Hanzlik, S. Heidman, and D. Smith, *Biochem. Biophys. Res. Commun.*, 1978, **82**, 310.
6 P. M. Dansette, V. B. Makedonska, and D. M. Jerina, *Arch. Biochem. Biophys.*, 1978, **187**, 290.
7 G. Bellucci, G. Berti, G. Ingrosso, and E. Mastrorilli, *J. Org. Chem.*, 1980, **45**, 299.
8 R. N. Armstrong, W. Levin, and D. M. Jerina, *J. Biol. Chem.*, 1980, **255**, 4698.
9 G. Bellucci, G. Berti, R. Bianchini, P. Cetera, and E. Mastrorilli, *J. Org. Chem.*, 1982, **47**, 3105.
10 G. Bellucci, G. Berti, M. Ferretti, F. Marioni, and F. Re, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 838.
11 G. Bellucci, G. Berti, G. Catelani, and E. Mastrorilli, *J. Org. Chem.*, 1981, **46**, 5148.
12 G. Berti, G. Catelani, M. Ferretti, and L. Monti, *Tetrahedron*, 1974, **30**, 4013.
13 T. Watabe, N. Ozawa, and K. Yoshikawa, *Biochem. Pharmacol.*, 1981, **30**, 1695.
14 J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.
15 P. A. Finan and C. D. Warren, *J. Chem. Soc.*, 1962, 2823.
16 B. Iselin and T. Reichstein, *Helv. Chem. Acta*, 1944, **27**, 1146.
17 F. Micheel, *Ber.*, 1930, **63**, 347.

Received 15*th April* 1983; *Paper* 3/598