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PREPARATION OF ISOBUTYL 3,4-ANHYDRO-2,6-DIDEOXY-DL-α-LYXO-HEXO-PYRANOSIDE AND ITS KINETIC RESOLUTION WITH MICROSOMAL EPOXIDE HYDROLASE.

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Summary. Racemic isobutyl 3,4-anhydro-2,6-dideoxy-α-lyxo-hexopyranoside, **3**, was prepared starting from the cycloadduct between 3-buten-2-one and isobutyl vinyl ether, through a sequence involving hydroboration-oxidation, mesylation, anomeric equilibration, elimination and epoxidation. The intermediate isobutyl 2,3,4,6-tetradeoxy-α-3-DL-hexenopyranoside,**10** was not stable under the conditions used for its preparation (t-BuOK in DMSO), being converted in part into the corresponding 2-hexenopyranoside. Under the same conditions the -anomer of **10** was stable. Complete hydrolysis of DL-**3** with aqueous NaOH, or with microsomial epoxide hydrolase (MEH) gave exclusively the xylo-diol (isobutyl DL-α-boivinopyranoside **5**). When the hydrolysis with MEH was stopped near 50% conversion and the product diol was separated from the unchanged epoxide, both were optically active, the former having the L and the latter the D configuration. An ee of at least 96% was found for both the diol and the epoxide by the use of a chiral shift reagent.

Previous work had shown that microsomial epoxide hydrolase (MEH), an important xenobiotic metabolizing enzyme,[1] is very efficient as a kinetic resolving agent for racemic isobutyl 3,4-anhydro-2,6-dideoxy-β-lyxo-hexopyranoside **1**,[2] and for the corresponding β-ribo-glycoside **2**,[3] giving access to the D and L forms of the xylo- and arabino-diols β-**5** and β-**6** (isobutyl β-boivinopyranoside and β-olivopyranoside) of high optical purities.

As an extension of our study of the relations between relative configuration of deoxy-anhydrosugars and enantioselectivity of MEH in their hydrolysis, it was of some interest to investigate the enzymatic hydrolysis

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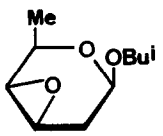
of the α-lyxo-epoxide **3**.



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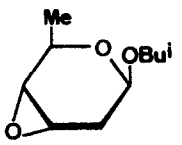

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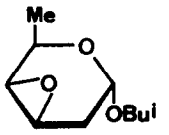

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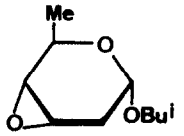

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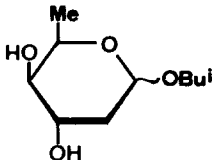

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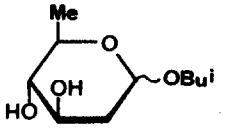

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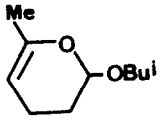
Preparation of the substrate

All compounds of this series can be prepared from the hydroboration-oxidation products of the cycloadduct **7** of 3-buten-2-one and isobutyl vinyl ether, a mixture of isobutyl β- and α-amicetosides **8**.[2] Their conversion into the mesylates **9**, followed by base-catalyzed elimination gives the unsaturated



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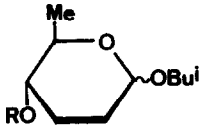
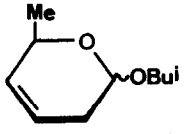


8:R=H

9:R=Ms



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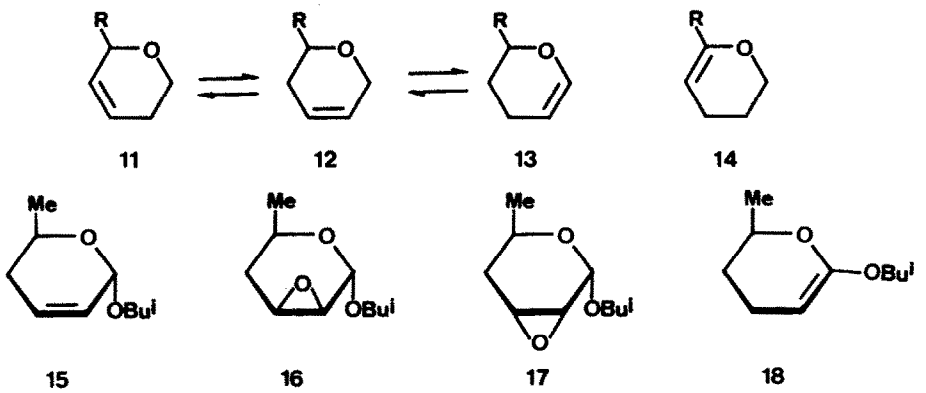
compounds **10**, that are epoxidized to **1-4**. Since the hydroboration product **8** consists of a 75:25 mixture of β- and α-anomers, access to the α-series is limited by the small amount of α-anomer present in this mixture. It therefore would be convenient to subject the crude mixture, prior to separation, to an acid-catalyzed anomeric equilibration. However the direct equilibration of the mixture of the anomers of the alcool **8** is not satisfactory, owing to the simultaneous formation in the α-pyranoside enriched product of substantial amounts of α- and β-furanosides.[4] A better procedure is to convert the unseparated mixture of **8** anomers into the corresponding mesylates

β- and α-**9**, which on heating with isobutyl alcohol in the presence of p-toluenesulfonic acid, or of a sulfonic resin yields β- and α-**9** in an inverted ratio of 15:85. A flash chromatography provides pure α-**9**.

The conversion of the mesylate α-**9** into the tetradeoxy-3-hexenopyranoside **10** was rather unsatisfactory. It had been previously found for β-**9** that the yield of the elimination reaction, when t-BuOK in DMSO was used as the base, did not exceed 50%, since a competing transesterification reaction converted part of the mesylate back into the free alcool β-**8**.[2] The same side-reaction has now been observed for the mesylate α-**9**. A similar tendency to the formation of relevant amounts of alcoholysis products has been reported for the reaction of 3-β-cholestanyl mesylate with t-BuOK in DMSO/benzene, a much improved olefin/alcohol ratio being however found when the corresponding tosylate was subjected to the same treatment.[5] In the case of α-**9** we did not achieve any improvement in passing from the mesylate to the tosylate ester. A further attempt to limit alcoholysis was made by using the tripsylate (2,4,6-triisopropylbenzenesulfonate) ester of α-**8**, in the expectation that this particularly bulky aryl group could hinder attack by the t-butoxy anion at sulfur. Unfortunately this hindrance was found to extend also to the attack at the H(3), so that the tripsylate ester was recovered mostly unchanged, even under forcing conditions.



The fact that the free alcohol α-**8** was easily recovered from the reaction mixture and amenable to reconversion into α-**9** somewhat limited the negative impact of transesterification on the yield of the elimination reaction. A second, more serious complication was in the fact that the product α-**10** was unstable in the t-BuOK/DMSO medium, and TLC showed that it was converted into a compound with a slightly higher R_F value. Since it has been reported [6] that

high temperature treatment of simple 2-alkyldihydropyrans with t-BuOK causes an equilibration between the isomeric species **11-13** (not **14**), the observed reaction was likely to involve conversion of **α-10** into its isomer **15**. Separation of **15** from **α-10** was difficult, owing to the limited differences in chromatographic retention times and to the high volatility of thesecompounds, causing substantial losses during solvent evaporations. It was thus found more convenient to dilute crude reaction solutions with water, extract with methylene chloride and add m-chloroperbenzoic acid directly to the thoroughly washed organic layer, in order to convert the hexenopyranosides into the corresponding epoxides, that are less volatile and usually highly amenable to chromatographic separation on silica.

Although the crude epoxidation product contained as much as five components, the alcoholysis product **α-8** and four epoxides, a single pass through a silica column allowed complete separation of **α-8** and of the main epoxides **3** and **16**, only the minor epoxides **4** and **17** being obtained as a mixture.

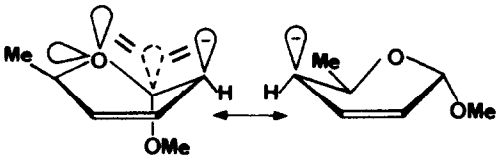
The structure of **3** was clearly demonstrated by its ^{1}H NMR spectrum that closely resembled that of the corresponding methyl glycoside,[7] the signals for H(1) (a double doublet, J = 4.5 and 1 Hz) and H(5) (a double quartet with J = 6.7 and ~1 Hz) being of particular diagnostic value. The structure of **16** was similarly proven, thus confirming the formation of **15** in the elimination reaction on **α-9**. The 2,3 position of the epoxide ring was clearly demonstrated by the anomeric proton signal, a narrow singlet pointing to a very weak coupling (~ 0.3 Hz) with a single proton at C(2), in accordance with literature data on 2,3-anhydropyranosides with a trans arrangement between epoxide oxygen and anomeric group for which $J_{1,2}$ is usually reported to be close to zero.[8] The multiplet shape of the H(5) signal (J = 6.2, 5.1 and 10 Hz) provided further confirmation for structure **16**.

Although the remaining two minor epoxides **4** and **17** were only obtained as a mixture, their anomeric proton signals were well separated and had the expected shapes: a double doublet (J = 4 and 3.8 Hz) for **4**, and a doublet (J = 2.8) for **17**. The shapes of the H(5) signal confirmed the structures, too.

The isomerization of **α-10** into **15** apparently is not accompanied by double bond shifts to either the 4,5 position (no **7** was detected), or to the 1,2-position. However, although no product identifiable as **18** was detected, the likely low stability of this ketene acetal in the reaction medium, would make its isolation quite problematic. The failure in forming double bond isomers involving the substituted positions α to the dihydropyran ring oxygen agrees with the reported absence of isomer **14** in the **11-13** equilibrium mixture.[6]

An interesting point is the absence of double bond migration of **β-10** formed in the t-BuOK/DMSO reaction of **β-9**.[2] Such a difference in reactivity between the α- and β-anomers is likely due to the easier abstraction of the H(2)

proton of α-**9** by the base to form the delocalized allylic carbanion **19**, responsible for the double bond shift. Such a preference could be rationalized on the basis of the known higher stability of carbanions having an electronegative substituent antiperiplanar to the charged non-bonding orbital on carbon,[9] a condition that exists in the α, but not in the β anomer of **19**. In other words the non-bonding orbital of the C(2) carbanion is in the same spatial relationship with respect to the anomeric substituent as one of the ring oxygen non-bonding orbitals. It can therefore be expected to exert a similar stabilizing effect on the α-anomer through n-σ^* overlap, such as



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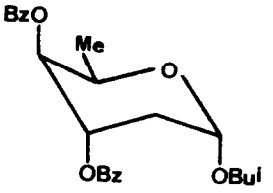
currently assumed as an explanation of the anomeric effect.[10] As a consequence of these complicating factors, the method that had proved to be acceptable for the preparation of **1** and **2**, turned out to be definitely less satisfactory for obtaining **3** and **4**. However, by limiting the duration of the elimination and epoxidation reactions (the rate of conversion into epoxide was lower for **15** than for α-**10**, owing to a lower nucleophilicity of the former caused by the vicinity of the ring oxygen to the double bond) it was possible to isolate pure **3** in about 20% overall yield from α-**9**.

Since our work had exploratory rather than preparative purposes, the search of better methods for obtaining DL-**3** was postponed to later investigations. During the epoxidation of α-**10**, **3** and **4** are formed in a ratio of about 4:1, a similar excess of **16** with respect to **17** being observed, too. This contrasts with the absence of diastereoface differentiation in the epoxidation of the β-**10** anomer.[2] Evidently an axial alkoxy group in the homoallylic position, or a pseudo-axial one in the allylic position shield the syn face of the double bond from the attack by the peracid more than a pseudo-equatorial or equatorial methyl in the allylic or homoallylic positions.

Alkaline hydrolysis of 3

In order to have a reference standard for the enzymatic hydrolysis experiments, **3** was subjected to hydrolysis in aqueous NaOH. A single diol was formed, identified as isobutyl 2,6-dideoxy-α-DL-xylo-hexopyranoside (isobutyl α-DL-boivinopyranoside,α-**5**) by ^{1}H NMR analysis of the corresponding

dibenzoate, **20**. The low values of $J_{1,2a}$, $J_{1,2e}$, $J_{3,4}$and $J_{4,5}$ were in good agreement with the triaxial structure expected for the $^4C_1$ conformer of **20**. These parameters are closely similar to those reported for the corresponding methyl glycosides.[11]



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The complete regiospecificity in the opening of the α-lyxo epoxide **3** at C(3) is paralleled by the same behaviour of the β-lyxo epoxide **1**, that is hydrolyzed exclusively to β-**5**,[2] and by the reaction of the α-lyxo methyl glycoside corresponding to **3**, that reacts with ammonia and with dimethylamine to give regiospecifically the xylo amino alcohols.[7] However, in the latter case, selectivity of attack is lower when azide or methoxy anions are used as nucleophiles: xylo and arabino adducts are formed, respectively in 90:10 and 80:20 ratios. A high preference for nucleophilic attack at C(3) of the more stable $^0H_1$(D) conformers of both **1** and **3** is expected since it involves the usual diaxial opening of the epoxide ring at the position that is more distant from the pyranose ring oxygen and therefore less influenced by its unfavourable inductive effect.[12] A somewhat smaller regiospecificity could be expected for **3** than for **1**, because of a possible shielding effect to the nucleophilic attack by the axial alkoxy group of the former, and this may explain the lower regioselectivity observed with some nucleophiles,[7] even if the different behaviour between ammonia, amines and hydroxide on one hand, and azide and methoxy on the other are not easily rationalized.

Enzymatic hydrolysis

The racemic epoxide **3** was subjected to complete hydrolysis in the presence of rabbit liver microsomes. As expected from the known fact that MEH acts through a base catalyzed nucleophilic attack by water and that the regioselectivity of this enzymatic ring opening reaction is usually similar to that observed in base, rather than in acid promoted hydrolyses,[13] only one diol was formed, the xylo derivative DL-α-**5**. No gas-chromatographically revealable trace of the alternative arabino-diol α-**6** was detected. As shown in the Figure, the enzymatic reaction was fairly rapid up to 50%

conversion,but slowed down considerably afterwards, a clear indication of a specific recognition of one enantiomer by the enzyme. This was confirmed in

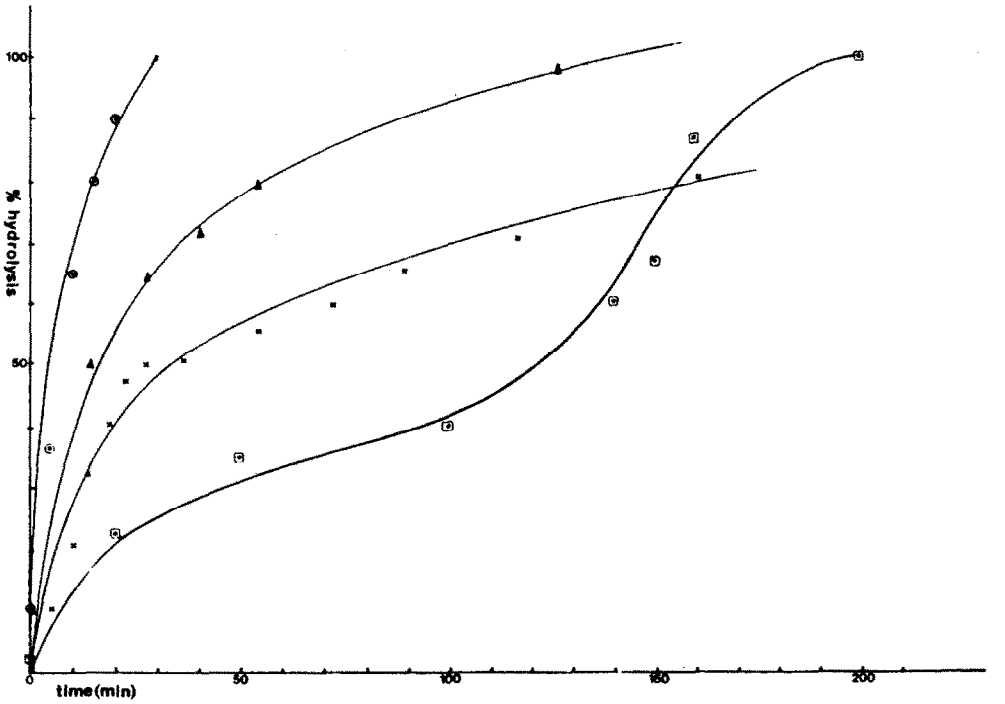


Figure. Enzymatic hydrolysis of DL-1 and DL-3 at constant microsome and different substrate concentrations. DL-1: ⊙ 0.01 M; ⊡ 0.05 M. DL-3: ▲ 0.0055 M; × 0.015 M

preparative runs, in which a higher substrate/microsome ratio was used, incubation was stopped around 50% conversion and the unchanged epoxide and the product diol were recovered and separated. Both were optically active, the diol being levorotatory and the epoxide dextrorotatory, which corresponds to an L configuration of α-5 and to a D configuration of 3.

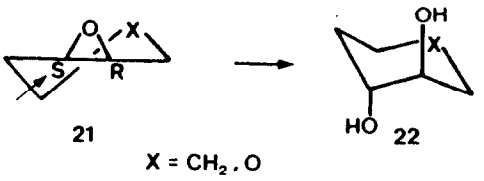
Enantiomeric excesses were determined by using the chiral shift reagent $Eu(hfc)_3$, whose addition to $CDCl_3$ solutions of either α-5 or 3, neatly separated the Me(6) doublets corresponding to their enantiomeric forms. It was thus established that the products of this enzymatic kinetic resolution were both optically pure within the limits allowed by this NMR analytical method. A cautious evaluation of these limits sets the minimum ee values for both 3 and α-5 at least at 96%, a remarkable result for a kinetic resolution.

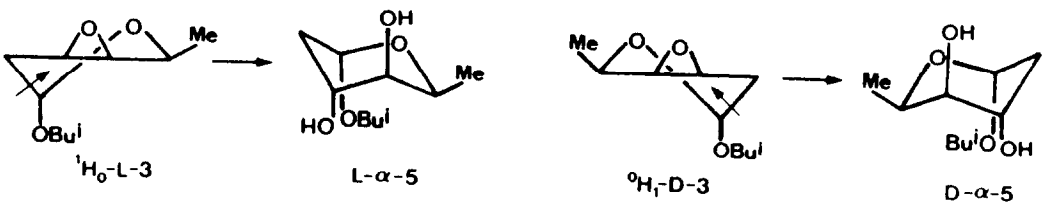
The result of the enzymatic hydrolysis of 3 agrees with previous interpretations,[14] according to which in epoxycyclohexanes and 3,4-epoxytetrahydropyrans there is a definite preference for the substrate to

adopt in the active site that conformation in which the six-membered ring exhibits the helicity shown in **21**, designated as 3,4M in epoxycyclohexanes, 1,2M in 3,4-epoxytetrahydropyrans and $^{1}H_{0}$ in α-L-**3**. A diaxial opening of the oxirane ring occurs by attack of water at the (S) carbon to give an (R,R)-diol **22**. Furthermore, a lipophilic substituent on the six membered ring appears to fit better into the enzyme active site when it is situated to the right of the epoxide ring viewed with the oxirane oxygen at the front upside. The L enantiomer of **3** meets all these requirements and is hydrolyzed by MEH in a highly preferential way with respect to its D antipode. The steric course of the reaction of DL-**3** with MEH therefore is similar to that previously observed for the β anomer DL-**1**,[2] but reaction rates differ significantly, as apparent from the biphasic curves shown in the Figure in which the previously obtained data for an analytical and a preparative run on DL-**1** are also shown.[2] During the first phase of the hydrolytic reaction, in which only the L enantiomer reacts, the rate of hydrolysis of **3** is somewhat slower than that of **1**. After 50% conversion a large decrease in rate is observed for **3**, an increase for **1**. These different types of kinetic behaviour had been observed before. The biphasic curve with increase in rate after 50% conversion, previously observed with styrene oxides,[15] was explained by assuming that both enantiomers fit into the MEH active site and can be hydrolyzed, but the lower K_m of one of them causes it to act as a competitive inhibitor toward its enantiomer when the racemic mixture is used as the substrate. Only after the former has been hydrolyzed, the latter can react, and since it is released more rapidly from the active site (higher V_{max}) the rate increases. The biphasic curve with a decrease in rate after 50% conversion, as observed for DL-**3**, and previously with the 3-tert-butyl-1,2-epoxycyclohexanes [14b] and with the β-ribo-epoxide **2**,[3] implies a low K_m and a higher V_{max} for one enantiomer and a very poor fit in the active site for the other, with an overall decrease in catalytic constant. This difference in the kinetic behaviour of the anomers **1** and **3**, could be explained with the presence in the latter of the axial anomeric group that could interfere with the fit of the substrate or with the approach of the water molecule in the active site during the ring opening step, slowing down the reaction of the L enantiomer, and much more that of the D one. However the present comparison proves that both mechanisms of enantioselection can lead to highly satisfactory kinetic resolutions.

The samples of DL-**3** to be used for the enzymatic hydrolysis experiments had to be carefully purified. Initial runs had a considerable induction time before hydrolysis started, that disappeared with samples that had been subjected to a second chromatographic purification. The impurity was most likely a trace of the isomeric epoxide **16**, acting as a competitive inhibitor.

A control made on this compound revealed that its rate of enzymatic hydrolysis is much slower than that of L-3.





In conclusion, the present results confirm that MEH can be an excellent kinetic resolving agent for certain racemic anhydrosugars, allowing to obtain one enantiomer of the epoxide and one of the corresponding trans diol, both with a very high optical purity. Although its use in larger scale preparations has not yet been investigated, it may provide a useful approach to the synthesis of enantiomeric forms of anhydro- or deoxysugars and other biologically interesting epoxides and diols, provided a satisfactory preparative method is available for the racemic epoxide and inhibition by substrate or product diol do not interfere with scale-up.

EXPERIMENTAL

NMR spectra were recorded with a Varian CTF-20 spectrometer on $CDCl_3$ solutions (Me_4Si internal standard). GC analyses were run on a Dani 3800 and on a Perkin-Elmer Sigma 3B chromatograph, both equipped with flame ionization detectors, under the following conditions: A) 1.8-m glass column packed with 10% Carbowax 20M on 80-100 mesh silanized Chromosorb W, N_2 flow 20 ml/min, programmed temperature 130°C (3 min) to 200°C, 7 deg/min. B) 1.8-m glass column packed with 3% OV 17 on 80-100 mesh silanized Chromosorb W, N_2 flow 40 ml/min, temperature 160°C. C) 1.8-m glass column packed with 3% NPGS on 80-100 mesh silanized Chromosorb W, N_2 flow 30 ml/min, programmed temperature 140°C (0 min) to 200°C, 3 deg/min. Optical rotations were measured with a

Perkin-Elmer 241 polarimeter at 20 2°C. Analytical TLC was carried out on silica plates (Merck, PSC Fertigplatten Kieselgel 60 F_{254}), components being located by spraying with 10% ethanolic phosphomolybdic acid and heating. Silica gel (Merck, Kieselgel 60, 70-230 mesh) was used for column chromatography. Preparative HPLC was performed on a Waters 500A instrument with Prepack 500-Silica cartridges. $MgSO_4$ was used as the drying agent.

Isobutyl 2,3,6-trideoxy-4-O-mesyl-α-erytro-DL-hexopyranoside(α-9). The crude mixture of the mesylates **β-9** and **α-9** (10 g)[4] in dry isobutyl alcohol (200 ml) was heated at 70°C, in the presence of DOWEX 50 W (H^+) resin, that had previously been washed with isobutyl alcohol and dehydrated by azeotropic distillation with benzene. The equilibration was followed by TLC. After 7 h the resin was filtered off and washed with several portions of isobutyl alcohol. Evaporation of the alcohol *in vacuo* left an oily residue composed by **α-9** and **β-9** in a ratio of 85:15. (GLC, cond. C, relative retention times **α-9**/**β-9** 1:1.20)

A similar result was obtained when an 8% solution of HCl in isobutyl alcohol was used and the reaction was conducted at room temperature for 24 h. Work-up involved neutralization with 10% aqueous Na_2CO_3, filtration and evaporation.

Separation was carried out by preparative HPLC (eluant 98.5:1.5 CH_2Cl_2/AcOEt), that produced 5 g of pure **α-9**. A further amount of this product (1.5 g) was recovered by subjecting mixed fractions recovered from the preparative run to a second chromatography (same eluant) through an open silica column, thus bringing the total recovery of pure **α-9** to 76%. The ^1H NMR spectrum was identical with the previously reported one.[2]

Reaction of α-9 with t-BuOK. The mesylate **α-9** (2.6 g, 9.8 mmole) was dissolved in dry DMSO (23 ml) and t-BuOK (2.75 g) was slowly added. After 4 h of stirring at room temperature **α-9** had completely disappeared and products **α-10**, **15** and **α-8** had formed as shown by TLC (eluant 9:1 hexane/AcOEt, R_F values of **α-10**, **15**, **α-9** and **α-8** : 0.56, 0.50, 0.08 and 0.04, respectively). A longer reaction time increased the amount of **15** with respect to **α-10**, as shown by the changes in relative intensities of the corresponding spots. The crude reaction mixture was diluted with water (50 ml) and extracted with hexane (4x50 ml). The organic layer was washed repeatedly with water until GLC analysis showed that DMSO was completely eliminated (residual DMSO could interfere with the subsequent epoxidation by consuming peracid and giving dimethyl sulfone). The CH_2Cl_2 solution was dried and slowly distilled through a Vigreux column to reduce the volume to about 10 ml. GLC analysis showed that it was composed of about 73% of an undifferentiated mixture of **α-10** and **15**, and of 27% of **α-8** (GLC cond. A, relative retention times 1:3.50).

Isobutyl 3,4-anhydro-2,6-dideoxy-α-DL-lyxo-hexopyranoside (3) and isobutyl 2,3-anhydro-4,6-dideoxy-α-DL-lyxo-hexopyranoside (16). The crude concentrated solution obtained as described in the previous paragraph was diluted with CH_2Cl_2 (60 ml) and treated with 85% m-chloroperbenzoic acid (1.50 g, 7.4 mmole). The solution was stored at 5°C. TLC (9:1 hexane / AcOEt) showed that **α-10** reacted more rapidly than **15**. After 60 h both had disappeared, and three spots corresponding to **3** (R_F = 0.20), **16** (R_F = 0.26) and **4** + **17** (R_F = 0.14) were visible, beside one for **α-8**. The solution was washed with aqueous $NaHSO_3$ and $NaHCO_3$, and concentrated to a small volume through a Vigreux column. GLC analysis (cond. A) gave the following composition: **3** + **16** 50%, unseparated peak, **17** (8%), **4** (7%), **α-8** (35%), relative retention times 1.00 : 1.07 : 1.12 : 1.35. Chromatographic separation on a 60.5 x 3.5 cm silica column (9:1 hexane/AcOEt) gave **16** (183 mg, 10%), **3** (160 mg, 8.7 %), **17** + **4** (123 mg, 6.7%) and **α-8** (305 mg, 16.5%)

In order to minimize the formation of **16** and **17** the reaction sequence was repeated as above, but with a decrease in the duration of the treatment with t-BuOK to 2 h, when a small amount of unreacted **α-9** was still present. In the epoxidation step the reaction was stopped as soon as TLC showed that **α-10** had completely disappeared and a little **15** was still present. The isolated yield of **3** increased to 20%, while that of **16** decreased to 4%.

Products **3** and **16** were purified by Kugelrohr distillation (1 mm Hg, bath temp 105° C) for analysis. **3**: ($C_{10}H_{18}O_3$ calcd.: C, 64.5; H, 9.7. Found: C, 64.9; H, 9.2). ^{1}H NMR: δ 0.90 and 0.91 (6 H, 2 d, J = 6.5 Hz, $OCH_2\underline{C}H\underline{Me}_2$), 1.33 (3 H, d, J = 6.7 Hz, C(5)Me), 1.77 (1 H, m, $OCH_2\underline{CH}Me_2$), 1.99 (1 H, m, C(2)H ax.), 2.03 (1 H, m, C(2)H eq.), 3.02 (1 H, m, C(4)H), 3.12 and 3.42 (2 H, 2 dd, J = 9.5, 6.9, 6.3 Hz, $O\underline{CH_2}CHMe_2$), 3.30 (1 H, m, C(3)H), 4.07 (1 H, dq, J = 6.7, 1.5 Hz, C(5)H), 4.67 (1 H, dd, C(1)H) ppm.

16: ($C_{10}H_{18}O_3$ calcd.: C, 64.5; H, 9.7. Found: C, 64.5; H, 9.7.) ^{1}H NMR: δ 0.86 (6 H, d, J = 6.6 Hz, $OCH_2CH\underline{Me}_2$), 1.03 (3 H, d, J = 6.2 Hz, C(5)Me), 1.20 (1 H, m, $OCH_2\underline{C}H\underline{Me}_2$), 1.77 (2 H, m, C(4)H ax. + C(4)H eq.), 2.90 (1 H, d, J = 3.9 Hz, C(2)H), 3.19 and 3.45 (2 H, 2 dd, J = 9.4, 6.7, 6.3 Hz, $O\underline{CH_2}CHMe_2$), 3.25 (1 H, m, C(3)H), 3.78 (1 H, m, J = 6.2, 5.1, 10.0 Hz, C(5)H), 4.87 (1 H, s, C(1)H) ppm.

The following values were obtained from the NMR spectrum of the **4** + **17** mixture: **4**: ^{1}H NMR: δ 0.85 and 0.94 (6 H, 2 dd, J = 6.6 Hz, $OCH_2CH\underline{Me}_2$), 1.37 (3 H, d, J = 7.0 Hz, C(5)Me), 4.17 (1 H, dq, J = 7.0, 0.5 Hz, C(5)H), 4.70 (1 H, dd, J = 3.8, 4.0 Hz, C(1)H) ppm.

17: ^{1}H NMR: δ 0.91 (6 H, d, J = 6.5 Hz, $OCH_2CH\underline{Me}_2$), 1.13 (3 H, d, J = 6.3 Hz, C(5)Me), 3.98 (1 H, ddq, J = 6.3, 2.5, 0.5 Hz, C(5)H), 4.98 (1 H, d, J = 2.8 Hz, C(1)H) ppm.

Isobutyl 2,6-dideoxy-α-DL-xylo-hexopyranoside. (α-5) A mixture of DL-**3** (73 mg) and aqueous NaOH 1.5 N (0.5 ml) was heated at 95° C in a sealed vial for 24 h. The solution was neutralized with dilute acetic acid and extracted with $CHCl_3$ (4x10 ml). Evaporation of the dried extract gave a crude residue, in which a main component was present for more than 90% (GLC, cond. B). The peak corresponding to α-**6** was totally absent. Kugelrohr distillation (0.01 mm Hg, bath temp 195°C) gave pure α-**5** as a colorless oil (58 mg, 72% yield). ($C_{10}H_{20}O_4$ calcd.: C 58.8, H 9.8; found C 58.4, H 9.6). ^1H NMR: δ 0.93 (6 H, d, J = 6.5 Hz, $OCH_2CH\underline{Me}_2$), 1.25 (3 H, d, J = 6.6 Hz, C(5)<u>Me</u>), 1.69 (2 H, bs, 2 <u>OH</u>), 1.65-2.05 (3 H, m, C(2)$\underline{H}_2$ and $OCH_2\underline{CH}Me_2$), 3.15 and 3.49 (2 H, 2 dd, J = 9.3, 6.1 and 6.8, $O\underline{CH}_2CHMe_2$), 3.48 and 3.79 (2 H, 2 m, C(3)<u>H</u> and C(4)<u>H</u>), 4.25 (1 H, dq, J = 6.0 and 10 Hz, C(5)<u>H</u>), 4.88 (1 H, dd, J = 3.2 and 1.2 Hz, C(1)<u>H</u>) ppm.

α-**5** was converted into the corresponding dibenzoate **20** by heating with benzoyl chloride in pyridine 15 h at 65°C. After a preparative TLC (9:1 hexane/AcOEt) **20** was obtained as a colorless oil. ($C_{24}H_{28}O_6$ calcd.: C 69.9, H 6.8; found C 69.6, H 6.7). ^1H NMR: δ 0.86 and 0.90 (6 H, 2 d, J = 6.6 Hz, $OCH_2CH\underline{Me}_2$), 1.25 (3 H, d, J = 6.7 Hz, C(5)<u>Me</u>)), 1.84 (1 H, m, $OCH_2\underline{CH}Me_2$), 2.20 (2 H, m, C(2)<u>H ax.</u> + C(2)<u>H eq.</u>), 3.15 and 3.54 (2 H, 2 dd, J = 6.4, 9.2, 6.6, $O\underline{CH}_2CHMe_2$), 4.57 (1 H, dq, J = 6.7, 1.5, C(5)<u>H</u>), 4.96 (1 H, m, $W_{\frac{1}{2}}$ = 7 Hz, C(1)<u>H</u>), 5.15 (1 H, m, C(4)<u>H</u>), 5.30 (1 H, m, C(3)<u>H</u>), 7.46 and 8.12 (10 H, 2 m, aromatic protons) ppm. Chemical shifts and coupling constants values of α-**5** and **20** are very close to those reported for methyl 2,6-dideoxy-α-D-<u>xylo</u>-hexopyranoside and the corresponding dibenzoate.[11]

Enzymatic hydrolysis of DL-3. Liver microsomes were prepared from phenobarbital-pretreated male New Zealand white rabbits as previously described,[14a] suspended in 0.01 M Tris-HCl buffer (pH 9.00) to a protein concentration of ca. 15 mg/ml and used immediately, or stored at -40° C.

Incubations were carried out in a shaking thermostatted bath at 37° C and evolution of the reaction was followed by direct injection of 2 μl samples into the gas-chromatographic column (cond. B) in order to establish the diol/epoxide ratio, on the basis of calibration curves obtained with artificial mixtures dissolved in the above buffer.

The Figure shows the curves obtained by using 5.5 and 15 mM concentrations of DL-**3** in the microsomial suspension (respectively 6 and 30 ml). Hydrolysis slowed down considerably after 50% conversion and was complete after several hours. GLC analysis after complete disappearance of the epoxide **3** showed that the diol DL-α-**5** was the only product, the α-**6** isomer being totally absent. When samples of DL-**3** that contained trace amounts of the epoxide **16** (barely perceptible by GLC) were subjected to the enzymatic hydrolysis, an induction

period of ca. 30 min was observed, before α-5 appeared in GLC. Thereafter, hydrolysis took the normal course illustrated by the curves of the Figure. Such an induction period disappeared on more careful purification of DL-3.

An incubation of DL-16 (5.5 mg) with the microsomial dispersion (5.5 ml) showed that it took 30 h for its complete hydrolysis.

Further preparative incubations were carried out on 100 mg samples of DL-3 in 30 ml of microsomial suspension. When conversion reached 50 ± 2% (after about 30 min) the reaction was stopped by cooling at -10° C and addition of 15% $ZnSO_4$ (10 ml). The suspension was extracted with hexane (3x25 ml) and the dried extract was evaporated slowly at atmospheric pressure through a Vigreux column in order to avoid loss of the rather volatile epoxide. GLC showed that the residue contained none of the diol α-5. This residue was distilled in a Kugelrohr (0.5 mm Hg, bath temp 70°C) to give pure D-3, $[\alpha]_D$ +86° (c 1.1, $CHCl_3$). Literature value for the corresponding methyl 3,4-anhydro-2,6-dideoxy-α-L-lyxo-hexopyranoside: $[\alpha]_D$ -125°(c 1.1, $CHCl_3$)[7].

The aqueous phase, after the extraction of 3, was extracted again with ethyl acetate (3x50 ml) and the dried extract was evaporated in vacuo. After Kugelrohr distillation of the residue, the diol L-α-5 was obtained, $[\alpha]_D$ -85° (c 1.0, $CHCl_3$). Literature value for the corresponding methyl 2,6-dideoxy-α-D-xylo-hexopyranoside: $[\alpha]_D$ +128°(c 0.9, $CHCl_3$).[11] The NMR spectra of D-3 and L-α-5 were identical to those of the corresponding racemic compounds.

Enantiomeric excesses were determined with the chiral shift reagent tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium (III), $Eu(hfc)_3$, after having established on samples of racemic 3 and α-5 the optimal ratio between substrate and reagent for separation of the Me(6) doublets corresponding to the enantiomers. A spectral width of 1000 Hz for 8192 data points was used. With the epoxide DL-3 addition of 8.4 mg of $Eu(hfc)_3$ to 4,7 mg of substrate in 0.5 ml of $CDCl_3$ produced two well-separated doublets at δ 2.90 and 3.10 ppm. With DL-α-5, 3.5 mg of substrate and 8.8 mg of $Eu(hfc)_3$ gave two doublets at δ 3.81 and 4.12 ppm.

When this treatment was repeated with the D-3 recovered from the incubation only the doublet at δ 3.10 was visible, the one at δ 2.90 being totally absent. Similarly, in the shifted spectrum of L-α-5, only the signal at 4.12 ppm appeared.

In order to evaluate the sensitivity of the employed method, 2% of racemic 3 corresponding to 1% L-3 was added to the sample tube in which the ee of D-3 had been determined. The signal at 2.90 ppm was barely perceptible in the ground noise. However a second addition of 2% DL-3 gave a signal at 2.90 ppm three times more intense than the ground noise. A similar result was obtained with L-α-5. A cautious evaluation of these results gives 96% as the lower limit for the ee's of both D-3 and L-α-5.

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References

1) J. Seidegard and J. W. DePierre, Biochim.Biophys.Acta **1983**, 695, 251-270.
2) P.L. Barili, G. Berti, G. Catelani, F. Colonna, and E. Mastrorilli, J. Org.Chem. **1987**, 52, 2886-2892.
3) P.L. Barili, G. Berti, G. Catelani, F. Colonna, and E. Mastrorilli, Gazz.Chim.Ital. **1988**, 118, 391-395.
4) G. Berti, G. Catelani, F. Colonna, M. Ferretti, and L. Monti, Gazz. Chim.Ital. **1985**, 115, 85-90.
5) F. C. Chang, Tetrahedron Lett. **1964**, 305-309.
6) J. Delaunay, A. Lebouc, and O. Riobè, Bull. Soc. Chim. France **1979**, II, 547-551.
7) A. Martin, M. Pais, and C. Monneret, Carbohydr. Res. **1983**, 113, 189-192.
8) F. Sweet and R. K. Brown, Can. J. Chem. **1968**, 46, 707-713; M. Chmielewski, J.Mieczkowski, W. Priebe, and A. Zamojski, Tetrahedron **1978**, 34, 3325-3329; M. M. Abdel-Malik, Q. J. Peng, and A. S. Perlin, Carbohydr. Res. **1987**, 159, 11-23.
9) A. J. Kirby, The Anomeric Effect and Related Stereoelectronic Effects at Oxygen, Springer Verlag, Berlin, **1983**, p. 77.
10) Ref. 9, p.64.
11) M. Marek and J. Jary, Coll. Czechoslov. Chem. Commun. **1980**, 45, 2979-84.
12) G. Berti, G. Catelani, M. Ferretti, and L. Monti, Tetrahedron **1974**, 30, 4013-4020.
13) G. Bellucci, G. Berti, M. Ferretti, F. Marioni, and F. Re, Biochem. Biophys. Res. Commun. **1981**, 102, 838-844.
14) a) G. Bellucci, G. Berti, G. Ingrosso, and E. Mastrorilli, J. Org. Chem. **1980**,45, 299-303.. b) G. Bellucci, G. Berti, R. Bianchini, P. Cetera, and E.Mastrorilli, ibid. **1982**, 47, 3105-3112. c) G. Bellucci, G. Berti, G. Catelani, and E. Mastrorilli, ibid. **1981**, 46, 5148-5150.
15) T. Watabe, N. Ozawa, and K. Yoshikawa, Biochem. Pharmacol. **1981**, 30, 1695-1698. (1981); R.P. Westkaemper and R. P. Hanzlik, Arch. Biochem. Biophys. **1981**, 208, 195-204.