High-performance liquid chromatographic determination of the enantiomeric excess of 1,3-glyceryl diethers obtained by stereoselective catalytic reduction

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ABSTRACT

The degree of stereodifferentiation in the catalytic enantioselective reduction of some prochiral diethers of 1,3-dihydroxy-2-propanone was determined by high-performance liquid chromatography using the cellulose tris(3,5-dimethylphenylcarbamate) derivative Chiralcel OD as the chiral stationary phase. The method was validated and the analytical results evaluated with respect to the rotation values of the reduction products and those reported previously for the same intermediates prepared from chiral starting materials.

INTRODUCTION

An asymmetric synthesis of 1,3-glyceryl diethers (Fig. 1) by stereoselectively reducing the corresponding ketones has been reported [1]. The glyceryl ethers, useful building blocks for synthesizing the enantiomers of several biologically active compounds such as phospholipids, platelet activating factor (PAF) and β-blockers, are usually prepared from chiral starting materials (D-mannitol, L-serine, ascorbic and tartaric acids).

Alternative approaches are based on the stereoselective chemical [2] or biological [3] reduction of prochiral ketones or the enzymatic differentiation of the two enantiotopic oxymethylenic groups in prochiral 1,3-glyceryl diesters obtained from 1,3-dihydroxypropanone [4,5].

The comparative evaluation of the degree of enantioselectivity achieved with these different approaches is limited, however, by the heterogeneity of the procedures used to determine the enantiomeric composition of the products.

This is usually determined: (a) by comparing the optical rotations with those reported for the same substances in optically pure forms [2,4,6–9]; (b) by derivatization using an enantiomerically pure reagent and analysing the diastereomeric composition by chromatography or NMR spectroscopy [2–5, 10–12]; and (c) by NMR analysis in the presence of an optically active shift reagent [6,13].

All these methods are limited in terms of accuracy, precision and feasibility. Determining the optical purity with the reported optical rotations re-

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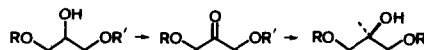

Fig. 1. Synthesis of 1,3-glyceryl diethers.

quires definite, high values of optical rotation as terms of reference. The second method is unsuitable for routine use and requires that the derivatization is quantitative with respect to the substrate and that the derivatizing agent is enantiomerically pure [14]. With the third procedure, high enantiomeric excesses cannot be determined with a satisfactory degree of precision [6].

Initially the degree of differentiation in the catalytic reduction was evaluated by comparing the optical rotation values of the hydrogenation products with those reported previously [6]. These compounds are usually prepared from 1,2-di-O-isopropylidene-sn-glycerol [11] and used as intermediates in the synthesis of optically pure PAF [6]. Later, however, considering the low values of the optical rotations and the limitations of the procedures, a high-performance liquid chromatographic method on a chiral stationary phase was used to determine the enantiomeric excesses. This technique allows the rapid, precise and direct determination of the enantiomeric excess. In this respect, cellulose tris-phenylcarbamates adsorbed on silica gel have proved to be chiral stationary phases suitable for the resolution of many racemic compounds. Secondary alcohols with aromatic rings, such as 1,3-glyceryl diethers 1–3 (Fig. 2), can interact with the polar carbamate groups via hydrogen bonding and with the phenyl groups via π–π interaction. The resulting diastereomeric complexes have comparatively different stabilities. Among those commercially available, one of the most effective is cellulose tris(3,5-dimethylphenylcarbamate) adsorbed on silica gel [15].

This paper describes the validated conditions used to resolve the enantiomeric components of three different 1,3-glyceryl diethers and discusses the results achieved by this procedure and by simply comparing polarimetric data.

EXPERIMENTAL

Apparatus and materials

High-performance liquid chromatography (HPLC) was carried out on a Chiralcel OD column (250 × 4.6 mm I.D.) from Daicel using a Waters 510 pump and a Pye Unicam Pu 4025 UV detector (analytical wavelength 254 nm). Chromatographic data were collected and processed on a Waters 740 data module. HPLC-grade solvents were purchased from Merck (Darmstadt, Germany).

Optical rotations were measured in a 1-dm cell of 1 ml capacity using a Perkin-Elmer 241 polarimeter.

Methods

The racemic compounds 1-O-benzyl-3-O-trityl glycerol (1), 1-O-octadecyl-3-O-trityl glycerol (2) and 1-O-benzyl-3-O-octadecyl glycerol (3) (Fig. 2), synthesized from 1,2-di-O-isopropylidene glycerol by the methods of Hirth and Barner [6], were oxidized to the ketones with pyridinium chlorochromate in dichloromethane and then stereoselectively reduced to the corresponding alcohols. The complexes utilized for the reduction were [(S)-(−)] and [(R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl]ruthenium(II) chloride dimers, triethylamine solvate, $\{Ru_2Cl_4[(S)\text{-}BINAP]_2\}N(C_2H_5)_3$ and $\{Ru_2Cl_4[(R)\text{-}BINAP]_2\}N(C_2H_5)_3$, prepared according to previously published methods [16].

In addition, the optically pure S- isomers of compounds 1 and 2 were obtained from 1,2-di-O-isopropylidene-sn-glycerol by the same method used for the preparation of the racemic forms.

The optical rotations of the hydrogenation products and of the S forms of compounds 1 and 2 were measured under the same conditions using the methods of Hirth and Barner [6].

The enantiomeric composition was determined by HPLC on a chiral stationary phase under the following conditions: compounds 2 and 3, hexane–isopropanol (99.4:0.6, v/v), flow-rate 1 ml/min;
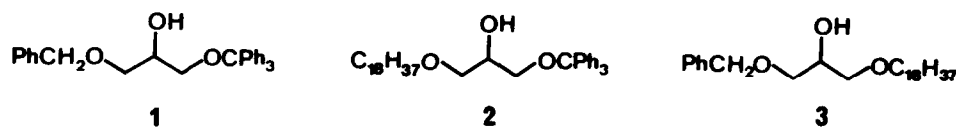
Fig. 2. 1,3-Glyceryl diethers prepared from 1,2-di-O-isopropylidene glycerol.
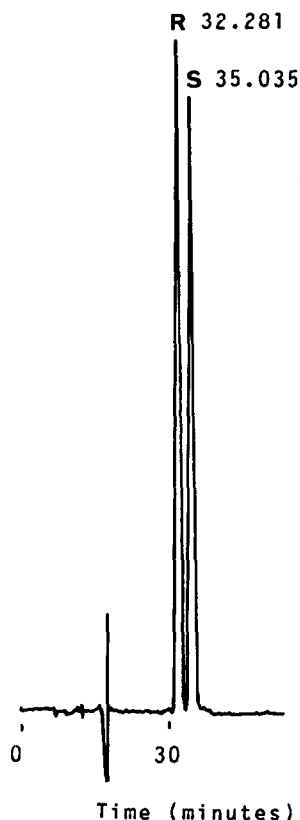
R 32.281

S 35.035

0   30

Time (minutes)

Fig. 3. Resolution of racemic 1-O-benzyl-3-O-trityl glycerol on the Chiralcel OD column. For the elution conditions, see under Experimental.

R 13.671

S 19.175

0   20

Time (minutes)

Fig. 4. Resolution of racemic 1-O-octadecyl-3-O-trityl glycerol on the Chiralcel OD column. For the elution conditions, see under Experimental.

compound 1, hexane–ethanol–water (90.63:9.06: 0.3, v/v/v), flow-rate 0.2 ml/min.

To achieve the resolution of compound 1 it was necessary to replace isopropanol with ethanol, to add water and to drastically reduce the flow-rate.

Figs. 3–7 show the chromatograms of the racemic compounds 1–3 and the S forms of compounds 1 and 2.

Table I gives the optical rotations of the hydrogenation products, the optical purities of the same compounds calculated from the reference values and the corresponding enantiomeric excesses determined by HPLC.

RESULTS AND DISCUSSION

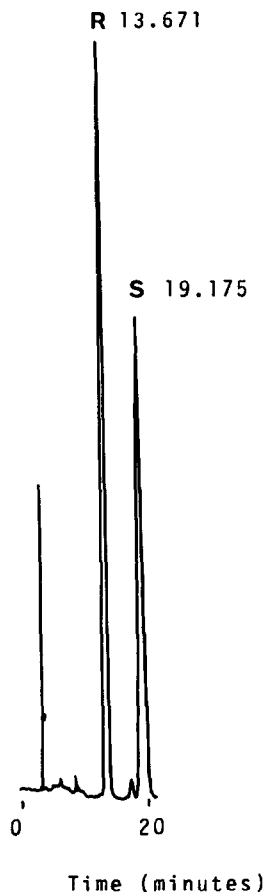The enantiomeric excesses determined by HPLC only agree with the optical purities calculated from

the optical rotations for compound 1, whereas they are significantly different for compounds 2 and 3.

When the stereodifferentiation of a reaction is high, as for the catalytic reduction to compounds 1 and 2, the amount of stereocontrol must be determined with the greatest precision possible. This is less essential for compound 3, as it was obtained with a low degree of enantioselectivity, usually less than 30% enantiomeric excess.

To confirm these results, optically pure (S)-1 and (S)-2 were prepared from 1,2-di-O-isopropylidene-sn-glycerol. A comparison of the chromatograms of optically pure (S)-1 and (S)-2 with those of racemic mixtures (see Figs. 6 and 7) clearly indicates that 1,2-di-O-isopropylidene-sn-glycerol provides optically pure compounds (S)-1 and (S)-2, as the minor
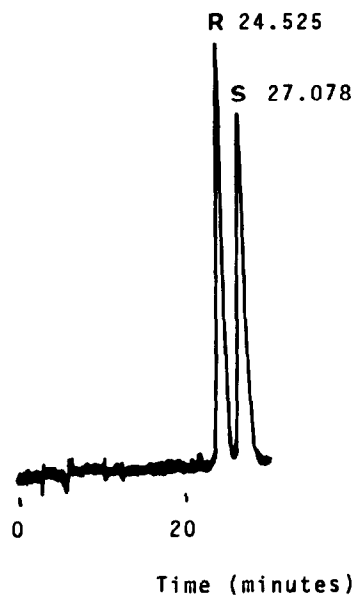
Fig. 5. Resolution of racemic 1-O-benzyl-3-O-octadecyl glycerol on the Chiralcel OD column. For the elution conditions, see under Experimental.
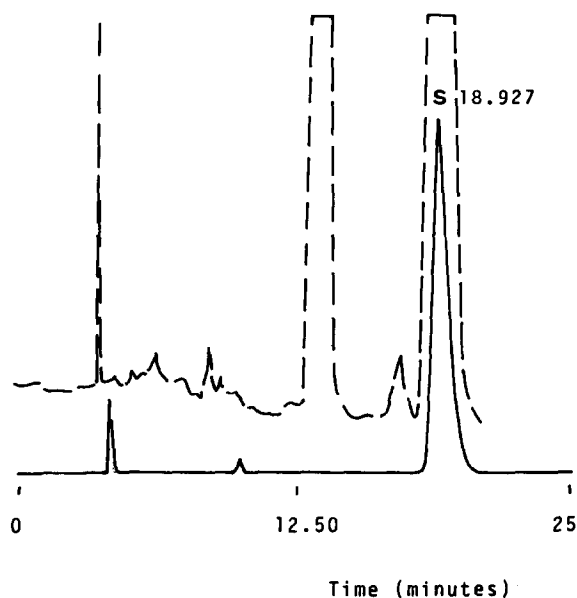

Fig. 7. Chromatogram of (S)-1-O-octadecyl-3-O-trityl glycerol (1-O-trityl-3-O-octadecyl-sn-glycerol) obtained with the Chiralcel OD column, superimposed over the chromatogram of the racemic mixture shown in Fig. 4 (broken line). For the elution conditions, see under Experimental.

Fig. 6. Chromatogram of (S)-1-O-benzyl-3-O-trityl glycerol (1-O-trityl-3-O-benzyl-sn-glycerol) obtained with the Chiralcel OD column, superimposed over the chromatogram of the racemic mixture shown in Fig. 3 (broken line). For the elution conditions, see under Experimental.
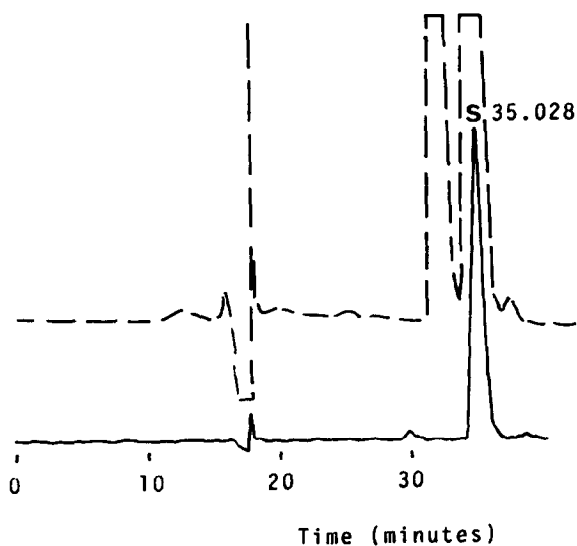
TABLE I

OPTICAL ROTATIONS, OPTICAL PURITIES CALCULATED FROM REFERENCE VALUES AND ENANTIOMERIC EXCESSES DETERMINED BY HPLC OF THE 1,3-GLYCERYL DIETHERS OBTAINED BY ENANTIOSELECTIVE HYDROGENATION

c = Concentration.

Compound	$[\alpha]_D^{20}$	Optical purity (%)[a]	Enantiomeric excess (HPLC) (%)
(R)-1	+5.59 (c = 5, benzene)	87.8	87.3
(S)-1	−5.54	87.0	86.0
(R)-2	+4.70 (c = 5, benzene)	98.3	88.5
(S)-2	−4.59	96.0	86.1
(R)-3	+0.60 (c = 10, benzene)	36.8	26.9
(S)-3	−0.48	30.1	20.6

[a] Reference optical rotation values [6]: −6.37 [(S)-1], −4.78 [(S)-2], + 1.63 [(R)-3].

R enantiomer is undetectable. The corresponding optical rotations are -6.50 and -5.31, respectively. The optical purity of stereoselectively hydrogenated products **1** and **2**, calculated with reference to these values agree with the enantiomeric excess values determined by HPLC. This proves that the relationship between the enantiomeric excess and the optical rotation for compounds **1** and **2** is linear. To determine the corresponding correlation coefficients (r), in addition to the two samples of compounds **1** and **2** obtained by catalytic hydrogenation and from optically active 1,2-di-O-isopropylidene glycerol, two samples of the compounds were prepared at different enantiomeric excesses by mixing the appropriate amounts of the enantiomerically pure S compounds with the racemic mixtures. The linear relationships found between the optical rotations and the corresponding enantiomeric excess values determined by HPLC gave $r = 0.99979$ and $r = 0.99795$ for compounds **1** and **2**, respectively.

The HPLC method based on the chiral stationary phase Chiralcel OD achieved the aim of developing a rapid, sensitive and precise procedure to determine the enantiomeric excess of these substrates. This is shown by the following method performances: linearity: $r = 0.99992$ (**1**), 0.99985 (**2**), 0.99944 (**3**); precision: determinations on six samples of the three racemic compounds at different concentrations showed a mean area first peak/area second peak ratio of 0.9896 (coefficient of variation 1.61%) for compound **1**, 1.0108 (1.86%) for compound **2** and 1.0016 (1.56%) for compound **3**; sensitivity: the method is sensitive to the injection on-column of about 20 ng of compound **1**, 0.2 μg of compound **2** and 1 μg of compound **3**. The calculated k' values gave separation factors of 1.15, 1.46 and 1.14 for compounds **1**, **2** and **3**, respectively.

The evaluation of the degree of stereodifferentiation based on the comparison of the polarimetric data implies increasing the reference rotation value of compounds **1** and **2** to 6.50 (0.05) and 5.31 (0.05), respectively.

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