Journal of Chromatography, 648 (1993) 119–129
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 351

Methods for the determination of the enantiomeric purity of the C_3-synthons glycidol (2,3-epoxy-1-propanol) and solketal [2,2-dimethyl-4-(hydroxymethyl)-1,3-dioxolane]

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(First received February 9th, 1993; revised manuscript received May 25th, 1993)

ABSTRACT

Accurate and reliable methods are presented for the determination of enantiomeric excess values of glycidol (2,3-epoxy-1-propanol), glycidyl esters, solketal [2,2-dimethyl-4-(hydroxymethyl)-1,3-dioxolane], homologous 1,3-dioxolane alcohols and substituted primary propanols in biological samples. One method consists of derivatization of the samples with 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate, followed by separation of the resulting diastereomers on a non-chiral reversed-phase (C_{18}) HPLC column. The second method uses direct injection of (aqueous) samples on to capillary GC columns coated with chiral stationary phases (2,3,6-tri-O-methyl-β- or a 2,3,6-tri-O-trifluoroacetyl-γ-cyclodextrin). The advantages and disadvantages of both methods are discussed.

INTRODUCTION

The enantiomers of glycidol (2,3-epoxy-1-propanol) and solketal [2,2-dimethyl-4-(hydroxymethyl)-1,3-dioxolane] have been advocated as attractive building blocks for the synthesis of homochiral pharmaceuticals and other specialty chemicals [1–4]. Since we have embarked on studies on the application of enzymes in kinetic resolution of these C_3-synthons [5–7], the changing ratio of the enantiomers had to be followed. This required methods that were able to determine the ratio from the start (racemic mixture) to the end (enantiomerically pure), that is, from an enantiomeric excess (e.e.) of 50 to 100%, in a reliable and accurate way. It should be stressed that in this context the sensitivity of the methods for the compound as such is of less importance.

Bioconversions are frequently carried out with either crude cellular material or with a small amount of purified enzyme in aqueous buffers. Hence, under these conditions, direct measurements of optical rotation in the medium are not reliable. Moreover, the determination of enantiomeric purity by NMR measurements in the presence of chiral shift reagents failed, because of instability of the products under these conditions. On the other hand, chromatographic methods could circumvent these restrictions if satisfactory accuracy and sensitivity could be achieved. A strategy frequently used in the analysis of racemic alcohols is to prepare diastereomers, *e.g.*, by using Mosher's acid [α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA)], and to separate the diastereomeric products by (non-chiral) GC or HPLC. Commercially available MTPA, however, is only 97.9–

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0021-9673/93/$06.00

99.8% pure and kinetic enrichment of one of the enantiomers has been reported [8]. Further, racemization can be expected under the acidic conditions, as observed here (see below) for trifluoroacetic anhydride. Hence, other derivatization methods seemed necessary to attain the high accuracy required.

Based on the derivatization procedure with 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate (TAGIT) described by Gal [9], diastereomers were prepared from glycidyl butyrate and they could be separated by HPLC (as reported in a preliminary communication by Philippi *et al.* [10]). Hence, it seemed worthwhile to investigate the reliability and accuracy of the method for glycidyl esters and glycidol, assuming that the derivatization proceeds as indicated in Fig. 1. To explore the scope of the method, other C_3-synthons and some homologous 1,3-dioxolane compounds with varying distance between the asymmetric carbon atoms were also investigated. For this derivatization, a modified route was devised, as indicated in Fig. 2.

Direct methods for the separation of enantiomers have been reported. König *et al.* [11] investigated trifluoroacetyl derivatives of epoxy alcohols (including glycidol) using GC with peralkylated α-cyclodextrins as a chiral stationary phase. Using α-, β- or γ-cyclodextrin stationary phases, many racemic compounds, including the trifluoroacetyl esters of glycidol and solketal, could be resolved [12]. Dougherty *et al.* [13] were able to separate glycidol without derivatization applying aqueous samples to a GC column with a permethyl-O-hydroxypropyl-α-

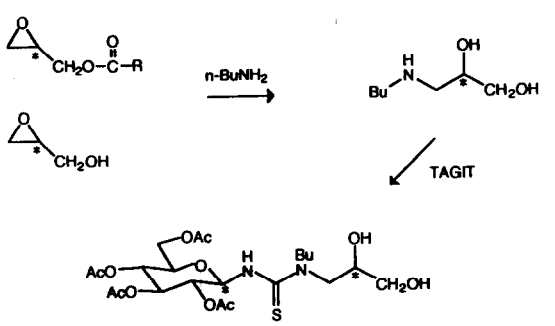


Fig. 1. Conversion of glycidol and glycidyl esters into their diastereomers with TAGIT.

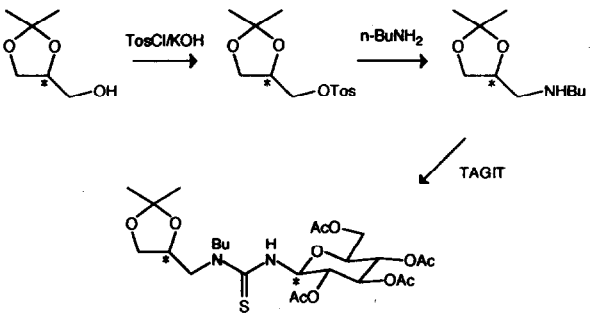


Fig. 2. Conversion of solketal into its diastereomers with TAGIT.

cyclodextrin stationary phase. As the latter strategy is very attractive for the analysis of samples resulting from biological conversions, GC on derivatized β- and γ-cyclodextrin stationary phases was also investigated for the separation of the enantiomers of glycidol, solketal and selected homologues.

EXPERIMENTAL

Chemicals

(*R*,*S*)-Glycidol, (*R*,*S*)-2-butanol, (*R*,*S*)-2-octanol, (*R*,*S*)-2,3-dibromo-1-propanol, (*R*,*S*)-2,3-dichloro-1-propanol, (*R*,*S*)-2-methyl-1-butanol and trifluoroacetic anhydride were purchased from Merck, (*R*)-(+)- and (*S*)-(−)-glycidol, (*R*)-(−)- and (*S*)-(+)-3-bromo-2-methyl-1-propanol, (*R*,*S*)-2-phenyl-1-propanol and (*R*,*S*)-glycerol formal from Aldrich, (*R*,*S*)-, (*R*)-(−)- and (*S*)-(+)-solketal, (*R*)-(−)- and (*S*)-(+)-2-butanol from Janssen Chimica, 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate (TAGIT), (*R*)-(−)- and (*S*)-(+)-2-octanol and (*R*,*S*)-3-buten-2-ol from Fluka and methanol (HPLC grade) from Rathburn; (*R*,*S*)- and (*R*)-(−)-glycidyl butyrate were gifts from Andeno. All other chemicals were of analytical-reagent grade (Merck).

Other glycidyl and solketyl esters [14], the homologous 1,3-dioxolane alcohols [15] and (*S*)-(−)- and (*R*)-(+)-2,3-O-isopropylidene glyceraldehyde (solketylaldehyde) [16,17] were synthesized according to the literature. The compounds had a chemical purity of 93–99.5%, as established by GC and ^{1}H NMR.

HPLC

Derivatization of glycidol and glycidyl esters. An aqueous sample, containing at least 5 mg of glycidol, was saturated with NaCl and extracted twice with an equal amount of dichloromethane. Samples containing a glycidyl ester were also extracted twice with dichloromethane. The extracts were dried with $MgSO_4$ and the solvent evaporated under reduced pressure. The residue was dissolved in 1 ml of *n*-butylamine and the solution was transferred into a 3-ml sample vial (Waters). The vial was tightly capped (PTFE septum) and heated in an oil-bath at 100°C for 1 h. Subsequently, the cap was removed to allow the residual amine to evaporate. The residue was stored overnight under reduced pressure in a desiccator over P_2O_5 to remove the last traces of the amine. The oily residue was dissolved in 200 μl of acetonitrile and 25 μl of this solution were mixed with 50 μl of acetonitrile containing 1 mg of TAGIT. After standing at room temperature for 5 min, a 5-μl sample of the mixture was injected into the HPLC system.

Derivatization of solketal and homologous 1,3-dioxolane alcohols. An aqueous sample, containing at least 1 mg of solketal or related alcohol, was extracted twice with an equal amount of dichloromethane. The combined extracts were dried with $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was dissolved in 10 ml of dry diethyl ether and the solution was transferred into a 50-ml flask. *p*-Toluenesulphonyl chloride (75 mg) and powdered KOH (500 mg) were added to the stirred solution at room temperature. After 15 min the mixture was poured into 20 ml of diethyl ether–water (3:1, v/v). After shaking, the organic layer was separated, washed twice with water and dried with $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in 2 ml of *n*-butylamine and placed in a 3-ml sample vial (Waters). The vial was tightly capped (PTFE septum) and the solution was heated in an oil-bath at 100°C for 1 h and then poured into 20 ml of a diethyl ether–water (3:1, v/v). After shaking, the organic layer was separated, washed four times with water and dried with $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in 0.5 ml of acetonitrile and 25 μl of this solution were added to 50 μl of acetonitrile containing 1 mg of TAGIT. After standing at room temperature for 5 min, 10 μl of this solution were injected into the HPLC-system.

Chromatography of the derivatives. The HPLC system consisted of a pump (Waters Model 510), an injector (Rheodyne Model 7125), a UV detector (Waters Lambda Max Model 480), an integrator (Waters data module) and a reversed-phase column (Waters Nova-Pak C_{18}, 100 × 8 mm I.D., particle size 4 μm). The column was eluted with aqueous methanol (compositions are given in Table I) at a constant flow-rate of 1.0 ml/min at room temperature. The eluent was monitored at 260 nm.

Preparation and identification of the diastereomers

Diastereomers of glycidol [N'-butyl-N'-(R,S)-2',3'-dihydroxypropyl(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiourea]. An 11-mg amount of racemic glycidol was derivatized as described above. The residue was dissolved in 250 μl of acetonitrile and 62 mg of TAGIT were added. After standing at room temperature for 60 min, the diastereomers were isolated by HPLC, as indicated above. The eluent was removed by evaporation under reduced pressure and the residues were stored overnight under reduced pressure in a desiccator over P_2O_5. ^{1}H NMR (400 MHz) was performed using a Varian VXR-400 S spectrometer. Well resolved spectra were obtained. From a comparison of the spectra of several 1-acylglycerol derivatives, the corresponding 2,3-epoxy-1-propanyl esters and the free alcohol, the presence of the 2,3-dihydroxy-1-propyl group could be deduced. The results are in agreement with the structure indicated in Fig. 1 and in line with that what could be predicted with analogous cases [9].

^{1}H NMR ($C^2H_3O^2H$): δ 5.90 (s, 1 H, glucose H-1α, J = 9.3 Hz), 5.33 (dd, 1 H, glucose H-3α, J = 9.3 Hz, J = 9.5 Hz), 5.12 (dd, 1 H, glucose H-2β, J = 9.5 Hz, J = 9.5 Hz), 5.03 (dd, 1 H, glucose H-4β, J = 9.7 Hz, J = 9.9 Hz), 4.20 (m, 1 H, NH), 4.31 (dd, 1 H, glucose H-6a, J = 12.3

TABLE I

CHROMATOGRAPHIC PARAMETERS ON A REVERSED-PHASE C_{18} HPLC COLUMN OF THE DIASTEREOMERS OBTAINED WITH TAGIT DERIVATIZATION OF GLYCIDOL, GLYCIDYL BUTYRATE, SOLKETAL, HOMOLOGOUS 1,3-DIOXOLANE ALCOHOLS AND SOME OTHER ALCOHOLS

α = Separation factor = $(t_2 - t_0)/(t_1 - t_0)$; R = resolution = $2(t_2 - t_1)/(W_1 + W_2)$, where W is the peak width. NR = Not resolved. The identity of the peaks (indicated with R or S, in parentheses) was derived from the results obtained with authentic compounds.

Compound	Mobile phase methanol–water (v/v)	Retention times of the diastereomers		α	R
		t_1 (min)	t_2 (min)		
Glycidol	50:50	29.3 (*R*)	31.7 (*S*)	1.09	1.40
Glycidyl butyrate	50:50	29.3 (*S*)	31.7 (*R*)	1.09	1.40
Solketal	58:42	38.3 (*R*)	40.4 (*S*)	1.06	1.01
Compound **1**	60:40	17.0		NR	NR
Compound **3**	60:40	10.8		NR	NR
Compound **4**	80:20	5.9		NR	NR
Compound **5**	60:40	58.6	74.2	[a]	[a]
		76.8	83.0		
Compound **6**	70:30	19.2	21.5	1.13	1.97
Compound **7**	80:20	8.4	9.4	1.15	1.75
Compound **8**	80:20	32.0		NR	NR
2,3-Dibromo-1-propanol	60:40	48.0		NR	NR
2-Butanol	80:20	7.5		NR	NR
2-Methyl-1-butanol	60:40	81.7		NR	NR

[a] Compound **5** consists of four diastereomers. Resolutions of the diastereomers are $R \geq 0.58$ and separation factors $\alpha \geq 1.04$ (which were calculated for peaks 2 and 3).

Hz, $J = 4.4$ Hz), 4.10 (dd, 1 H, glucose H-6b, $J = 12.3$ Hz, $J = 2.2$ Hz), 4.08 (dd, 1 H, glycerol H-1), 3.88 (ddd, 1 H, glucose H-5α, $J = 2.2$ Hz, $J = 4.4$ Hz, $J = 10.1$ Hz), 3.8 (m, 1 H, glycerol H-2), 3.72 (m, 2 H, butyl H-1), 3.66 (m, 1 H, glycerol H-1, $J = 5.1$ Hz, $J = 7$ Hz), 3.55 (dd, 2 H, glycerol H-3, $J = 5.1$ Hz), 2.03 (s, 3 H, acetyl), 2.01 (s, 3 H, acetyl), 2.00 (s, 3 H, acetyl), 1.98 (s, 3 H, acetyl), 1.64 (m, 2 H, butyl H-2, $J = 7$ Hz), 1.33 (m, 2 H, butyl H-3, $J = 7.3$ Hz, $J = 7$ Hz), 0.96 (t, 3 H, butyl H-4, $J = 7.3$ Hz).

Diastereomers of solketal {N'-butyl-N'-[(R,S)-2',2'-dimethyl-1',3'-dioxolan-4'-yl]methyl(2,3,-4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiourea}. A 14-mg amount of racemic solketal was derivatized with 45 mg of TAGIT and the diastereomers produced were isolated as described above. Well resolved ^{1}H NMR spectra of the diastereomers were obtained. The presence of the (2,2-dimethyl-1,3-dioxolan-4-yl)methyl moiety could be deduced from the similarity of the chemical shifts and the coupling constants observed for the diastereomers and the free alcohol. The results are in agreement with the structure indicated in Fig. 2.

^{1}H NMR ($C^2H_3O^2H$): δ 5.97 (s, 1 H, glucose H-1α, $J = 9.1$ Hz), 5.35 (dd, 1 H, glucose H-3α, $J = 9.5$ Hz, $J = 9.5$ Hz), 5.18 (dd, 1 H, glucose H-2β), 5.03 (dd, 1 H, glucose H-4β, $J = 9.7$ Hz, $J = 10.0$ Hz), 4.40 (m, 1 H, dioxolane H-4), 4.32 (dd, 1 H, glucose H-6a, $J = 12.3$ Hz, $J = 4.2$ Hz), 4.11 (dd, 1 H, glucose H-6b, $J = 12.3$ Hz, $J = 2.4$ Hz), 4.10 (dd, 1 H, dioxolane H-5, $J = 8.3$ Hz, $J = 6.0$ Hz), 3.91 (ddd, 1 H, glucose H-5α, $J = 2.4$ Hz, $J = 4.2$ Hz, $J = 10.1$ Hz), 3.75 (m, 2 H, butyl H-1), 3.65 (dd, 1 H, dioxolane H-5, $J = 8.3$ Hz, $J = 7.3$ Hz), 3.63 (dd, 2 H, dioxolane H-4'),

2.02 (s, 3 H, acetyl), 2.01 (s, 3 H, acetyl), 2.00 (s, 3 H, acetyl), 1.99 (s, 3 H, acetyl), 1.59 (m, 2 H, butyl H-2, $J = 7$ Hz), 1.47 (s, 3 H, dioxolane H-2′), 1.33 (s, 3 H, dioxolane H-2″), 1.33 (m, 2 H, butyl H-3, $J = 7.3$ Hz, $J = 7$ Hz), 0.95 (t, 3 H, butyl H-4, $J = 7.3$ Hz).

Gas chromatography

Direct analysis. An aqueous sample (1 ml), containing *ca.* 1.0 mg of the compound to be determined, could be used directly for analysis (0.2–1.0 μl injected) or via extraction with 3.0 ml of dichloromethane. The dichloromethane extract was dried with $MgSO_4$ and the solvent was evaporated with a stream of nitrogen until a residue of *ca.* 50 μl remained.

Derivatization of the samples. An aqueous sample was extracted as described above. The dichloromethane extract was dried with $MgSO_4$ and the solvent was evaporated with a stream of nitrogen. For derivatization, the residue was dissolved in 0.5 ml of dry diethyl ether and 100 μl of trifluoroacetic anhydride (TFAA) were added. After standing at room temperature for 30 min, the excess of TFAA, the trifluoroacetic acid produced and the solvent were removed with a stream of nitrogen and the residue was dissolved in 50 μl of dichloromethane.

Chromatography of the compounds and derivatives. Chromatography was performed with a Hewlett-Packard Model 5890 Series II gas chromatograph with a split injector, a flame ionization detector and an integrator (Hewlett-Packard 3365 Chemstation). The apparatus was equipped with a CP-cyclodextrin-2,3,6-M-19 capillary column (25 m × 0.25 mm I.D., film thickness 0.25 μm) (Chrompack, Middelburg, Netherlands) or a Chiraldex G-TA capillary column (20 m × 0.25 mm I.D., film thickness 0.125 μm) (Astec, Whippany, NJ, USA). The latter was preceded by a retention gap (2.5 m × 0.32 mm I.D.). Nitrogen was used as the carrier gas and the inlet pressure was 56 kPa for the first and 25 kPa for the second column. A split flow of 100:1 was used. The injector and detector temperatures were kept constant at 200 and 250°C, respectively. The injection volume of the samples was 0.2–1.0 μl.

RESULTS AND DISCUSSION

Reversed-phase HPLC of the diastereomers

Derivatization with TAGIT has been applied to the determination of the enantiomeric purity of α-amino acids [18–20], amphetamines [21], catacholamines [22–24], epoxides [9], amino alcohols [25,26], α-methylamino acids [27] and some pharmaceuticals [28–31]. As reported in a preliminary communication [10], derivatization of glycidyl butyrate with TAGIT, followed by reversed-phase HPLC on a C_{18} column, gave satisfactory resolution for determination of the enantiomeric excess (e.e.) values in the enzymatic hydrolysis of glycidyl butyrate. Therefore, we first attempted to determine the reliability and accuracy of the method for a number of glycidyl esters and glycidol itself.

As the derivatization procedure has to be executed in a water-free system, extraction was required. The extracted glycidol or glycidyl ester appeared to be converted within 1 hour into the corresponding amino alcohol (Fig. 1). Treatment of this with TAGIT gave the diastereomer within 5 min, as judged from analysis by HPLC. Nearly baseline separation [$R = 1.40$; $\alpha = 1.09$ (Table I)] was achieved with HPLC (Fig. 3). Several control experiments in which the presence of protein and phosphate buffers (0.1 *M*, pH 6–8) in the sample was tested were carried out. No

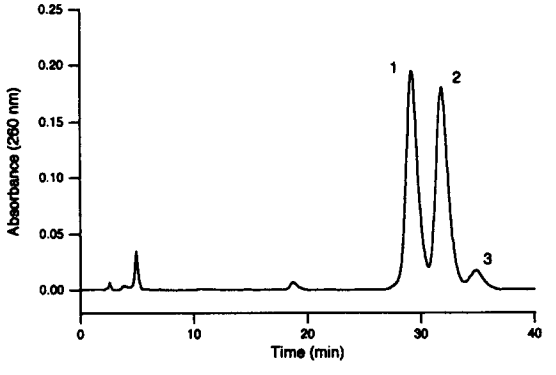


Fig. 3. Chromatogram of TAGIT-derivatized racemic glycidol on a reversed-phase C_{18} HPLC column. The peaks originate from (1) the diastereomer of (*R*)-(+)-glycidol, (2) the diastereomer of (*S*)-(−)-glycidol and (3) *n*-butylamine.

effect was found in samples containing enantiomerically pure (*R*)-(−)-glycidyl butyrate or racemic glycidol, as only one diastereomer and two diastereomers in a 1:1 ratio were found, respectively. This indicates that under the conditions used no racemization or kinetic enrichment occurs and that TAGIT is enantiomerically pure. As the esters and glycidol gave the same products with HPLC, apparently ester hydrolysis occurs and ring opening proceeds in the same way. *n*-Butylamine seemed to be the best choice for the first step in the procedure as isopropylamine, *tert.*-butylamine, pentylamine and both enantiomers of amphetamine gave diastereomers with less adequate separation. Aqueous samples containing glycidol in addition to a glycidyl ester could be analysed by making use of the large differences in solubility in organic solvents. For this purpose the sample was extracted with dichloromethane (no salt was added) and small amounts of glycidol in the organic solvent layer were removed by extraction with water. The water layer from the first extraction could be used for the determination of glycidol.

To determine solketal, the procedure had to be adapted as the compound as such does not react with the amine. Hence solketal was first tosylated and the product was subsequently derivatized with *n*-butylamine (Fig. 2), analogous to the procedure developed by Sowden and Fischer [32] for the synthesis of L-1-amino-2,3-propanediol from (*S*)-(+)-solketal. The amine products reacted readily with TAGIT and the diastereomers formed could be separated (Fig. 4), although the separation parameters [$R = 1.01$; $\alpha = 1.06$ (Table I)] were less satisfactory than for glycidol. Therefore, experiments were performed to check whether the method would be reliable at high e.e. values. In a typical experiment, enantiomerically pure (*S*)-(+)-solketal was deliberately contaminated with 0.25% of the *R*-isomer. It appeared that the experimentally determined e.e. value (99.4 ± 0.2%; six measurements) was very close to the theoretical value (99.5%). As with glycidol, control experiments indicated that no racemization or kinetic enrichment occurs in the procedure developed for solketal.

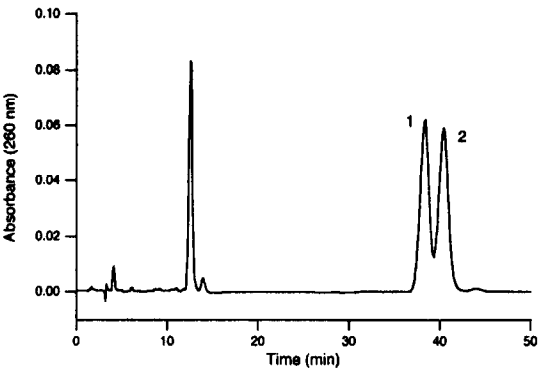
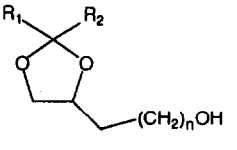


Fig. 4. Chromatogram of TAGIT-derivatized racemic solketal on a reversed-phase C_{18} HPLC column. Peak 1 originates from the diastereomer of (*R*)-(−)-solketal and peak 2 from the diastereomer of (*S*)-(+)-solketal.

To investigate the scope of the method, a number of homologous 1,3-dioxolane compounds (Fig. 5) and 2-butanol, 2-methyl-1-butanol and 2,3-dibromo-1-propanol were subjected to the derivatization procedure developed for solketal. Only the homologous 1,3-dioxolane compounds with the hydroxy group next to the chiral centre at C-4 ($n = 0$; compounds **5**, **6** and **7**) gave diastereomeric products that could be separated. Bulky substituents (R_1 and R_2) as present in compounds **6** and **7** caused an increase in the retention time and a better resolution of the diastereomeric products (Table I). Glycerol formal (compound **1**), which lacks the two methyl groups of solketal at C-2, gave unsatisfactory results. In theory, the introduction of a



Compound	n	R_1	R_2
1	0	H	H
2	0	CH_3	CH_3
3	1	CH_3	CH_3
4	3	CH_3	CH_3
5	0	CH_3	C_3H_7
6	0	-$(CH_2)_4$-	
7	0	-$(CH_2)_5$-	
8	3	-$(CH_2)_5$-	

Fig. 5. Structures of homologous 1,3-dioxolane alcohols. **1** = 4-(Hydroxymethyl)-1,3-dioxolane (glycerol formal); **2** = 2,2-dimethyl-4-(hydroxymethyl)-1,3-dioxolane (solketal); **3** = 2,2-dimethyl-4-(2-hydroxyethyl)-1,3-dioxolane; **4** = 2,2-dimethyl-4-(4-hydroxybutyl)-1,3-dioxolane; **5** = 2-methyl-2-propyl-4-(hydroxymethyl)-1,3-dioxolane; **6** = 2,2-butylene-4-(hydroxymethyl)-1,3-dioxolane; **7** = 2,2-pentylene-4-(hydroxymethyl)-1,3-dioxolane; **8** = 2,2-pentylene-4-(4-hydroxybutyl)-1,3-dioxolane.

second chiral centre should lead to four different diastereomeric products. Four peaks were indeed present in the chromatogram of compound **5** (second chiral centre at the C-2 atom) in the expected region (Fig. 6). Their assignment has not been carried out. The diastereomers of the homologous 1,3-dioxolane compounds with $n > 0$ (Fig. 5) and of the other alcohols showed no separation on the column used.

For the procedures described here, the samples should contain at least 1 mg of solketal or 5 mg of glycidol and 5–10 μg of the diastereomers were used for HPLC. Hence, substantial improvements could be made with respect to sensitivity, *e.g.*, by improving the yield of extraction (solketal and glycidol show good solubility in water). As our objective was to develop a reliable and sensitive method to determine the ratio of the enantiomers, no attempts were made in this direction.

Gas chromatography on chiral stationary phases

The enantiomeric composition of glycidol- or solketal-containing samples was determined using two commercially available capillary GC columns containing 2,3,6-tri-O-methyl-β-cyclodextrin (β-CD) or 2,3,6-tri-O-trifluoroacetyl-γ-cyclodextrin (γ-CD) as chiral stationary phase. It appeared that aqueous samples of glycidol and solketal could be injected directly on to the capillary columns, giving satisfactory separation

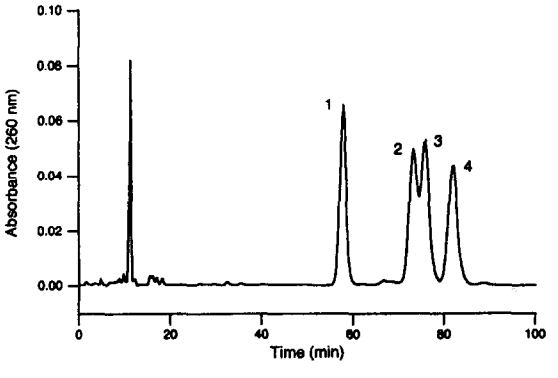


Fig. 6. Chromatogram of TAGIT-derivatized racemic compound **5** [2-methyl-2-propyl-4-(hydroxymethyl)-1,3-dioxolane] on a reversed-phase C_{18} HPLC column. Peaks 1–4 originate from the four diastereomeric products, but their assignment has not been carried out.

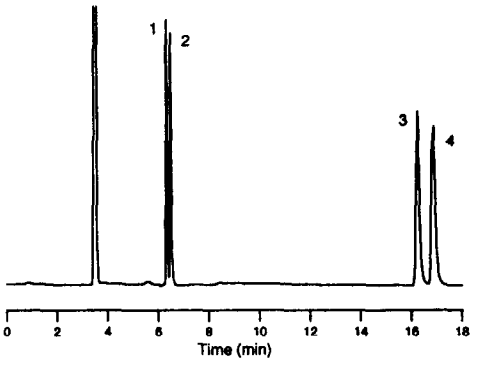


Fig. 7. Chromatogram of racemic glycidol and racemic solketal on a capillary GC column with a 2,3,6-tri-O-trifluoroacetyl-γ-cyclodextrin stationary phase at a column temperature of 90°C. The peaks originate from (1) (*R*)-(+)-glycidol, (2) (*S*)-(−)-glycidol, (3) (*R*)-(−)-solketal and (4) (*S*)-(+)-solketal.

(Fig. 7; Tables II and III), the γ-CD column giving a better separation than the β-CD column. However, to avoid contamination of the system with proteins and buffer compounds (deterioration of the column was observed after *ca.* 50 direct injections), extraction was routinely applied. Owing to their water solubility, samples had to contain *ca.* 1 mg of the compound under these conditions. To check the reliability and accuracy of the method, control experiments were performed. In a typical experiment, to enantiomerically pure (*S*)-solketal 0.25% of the *R*-isomer was added. Experiments on the γ-CD column (six measurements) gave an e.e. value of $99.52 \pm 0.07\%$, very close to the theoretical value of 99.50%.

To investigate which compounds could be determined, racemic mixtures of glycidyl esters, homologous 1,3-dioxolane compounds (Fig. 5), substituted primary propanols and some other alcohols were tested. The enantiomers of most of these compounds could be baseline separated ($R > 1.5$) using the γ-CD column (Table II). The resolution of the glycidyl esters, however, decreased with an increasing chain length of the carboxylic acid moiety. For carboxylic acids longer than C_5 separation was inadequate. Enantiomers of the homologous 1,3-dioxolane alcohols with the hydroxy group close to the chiral centre at C-4 ($n = 0$ and 1; compounds **1**, **3**, **6** and **7**) were all baseline separated. The enantio-

TABLE II

CHROMATOGRAPHIC PARAMETERS ON A CAPILLARY GC COLUMN WITH A CHIRAL (2,3,6-TRI-O-TRIFLUOROACETYL-γ-CYCLODEXTRIN) STATIONARY PHASE OF ENANTIOMERS OF GLYCIDOL, GLYCIDYL ESTERS, SOLKETAL, HOMOLOGOUS 1,3-DIOXOLANE COMPOUNDS AND SOME OTHER ALCOHOLS

Terminology and abbreviations as in Table I.

Compound	Temperature (°C)	Retention times		α	R
		t_1 (min)	t_2 (min)		
Glycidol	80	8.03 (*R*)	8.35 (*S*)	1.068	2.25
Glycidyl acetate	100	9.22	10.0	1.137	5.59
Glycidyl propionate	90	15.8	16.4	1.052	2.33
Glycidyl butyrate	100	14.8 (*R*)	15.1 (*S*)	1.030	1.63
Glycidyl pentanoate	85	47.1	48.0	1.021	1.26
Glycidyl hexanoate	90	65.0	65.8	1.014	0.85
Glycidyl octanoate	120	41.6		NR	NR
Solketal	90	16.2 (*R*)	16.9 (*S*)	1.048	2.34
Compound **1**	90	14.5	15.0	1.046	2.12
Compound **3**	100	17.9	18.4	1.037	1.85
Compound **4**	100	56.0		NR	NR
Compound **5**	100	23.7 [a]	24.9		
Compound **6**	100	45.3	46.8	1.036	1.96
Compound **7**	110	42.8	43.8	1.025	1.45
Compound **8**	150	22.9		NR	NR
Solketyl acetate	70	52.2	53.3	1.022	1.20
Solketyl butyrate	120	>60			
2,3-Dichloro-1-propanol	90	22.7	23.6	1.042	1.40
2,3-Dibromo-1-propanol	110	24.3	25.0	1.034	1.65
3-Bromo-2-methyl-1-propanol	90	22.2 (*R*)	22.8 (*S*)	1.032	1.32
2-Phenyl-1-propanol	100	38.7	40.3	1.045	2.49
2-Butanol	40	6.65 (*S*)	6.85 (*R*)	1.055	1.56
2-Octanol	60	39.8	40.5	1.019	1.15
3-Buten-2-ol	40	6.59	6.73	1.039	1.17

[a] Compound **5** consists of four diastereomers, but only two peaks were observed, with a ratio of 3:1 (peak 1:peak 2).

mers of the homologous 1,3-dioxolane alcohols with $n = 3$ (compounds **4** and **8**) were not resolved. Introduction of a second chiral centre at C-2 (compound **5**) leads theoretically to four diastereomers. However, only two peaks could be detected in the chromatogram. The ratio of the areas of peaks 1 and 2 is 3:1. Hence, the first peak probably represents three diastereomers. The number of compounds that could be determined using the β-CD column appeared to be considerably smaller (Table III). Enantiomers of glycidyl butyrate and of the three primary halopropanols tested were not resolved and in most other instances the chromatographic parameters were significantly below acceptable values. Only the enantiomers of the homologous 1,3-dioxolane compounds could be sufficiently separated, except those of compound **8**. Also, the four diastereomers of compound **5** were not detected. In the chromatogram only three peaks were observed, of which peak 1 may contain two of the diastereomers. The enantiomers of solketylaldehyde, products which are formed from racemic solketal in the conversion with alcohol dehydrogenases [7], gave a baseline separation with a very high resolution ($R = 3.32$).

Alcohols can be derivatized with TFAA prior to the GC analysis to improve their volatility [12,13,33]. To investigate whether this improved the resolution of the alcohols studied here, the

TABLE III

CHROMATOGRAPHIC PARAMETERS ON A CAPILLARY GC COLUMN WITH A CHIRAL (2,3,6-TRI-O-METHYL-β-CYCLODEXTRIN) STATIONARY PHASE OF ENANTIOMERS OF GLYCIDOL, GLYCIDYL BUTYRATE, SOLKETAL, HOMOLOGOUS 1,3-DIOXOLANE COMPOUNDS AND SOME OTHER ALCOHOLS

Terminology and abbreviations as in Table I.

Compound	Temperature (°C)	Retention times		α	R
		t_1 (min)	t_2 (min)		
Glycidol	45	22.1 (*R*)	22.9 (*S*)	1.037	1.52
Glycidyl butyrate	100	15.4		NR	NR
Solketal	95	15.5 (*R*)	15.9 (*S*)	1.028	1.43
Compound **1**	70	38.5	39.7	1.033	1.24
Compound **3**	110	15.4	15.9	1.034	1.47
Compound **4**	120	31.2	31.9	1.023	1.29
Compound **5**	120	13.4 [a]	14.0		
		14.4			
Compound **6**	125	20.0	20.5	1.030	1.54
Compound **7**	125	32.3	33.1	1.026	1.29
Compound **8**	150	52.2		NR	NR
Solketylaldehyde	90	7.18 (*S*)	7.67 (*R*)	1.097	3.32
2,3-Dichloro-1-propanol	100	18.1		NR	NR
2,3-Dibromo-1-propanol	125	17.2		NR	NR
3-Bromo-2-methyl-1-propanol	80	45.1	45.9	1.020	0.43
2-Phenyl-1-propanol	100	46.3	47.4	1.027	1.13
2-Butanol	40	7.16 (*R*)	7.37 (*S*)	1.041	1.16
2-Octanol	70	40.4	41.1	1.020	0.73
3-Buten-2-ol	45	5.70	5.91	1.056	1.21

[a] Compound **5** consists of four diastereomers. Three peaks were observed in a ratio of 2:1:1 (peak 1:peak 2:peak 3).

racemic compounds were derivatized with TFAA and injected on to the β-CD column. In general, the separation of the products could be performed at lower column temperatures and the peaks obtained showed less tailing when compared with the underivatized alcohols. The chromatographic parameters of almost all of the compounds tested improved (Table IV). For instance, the resolution of the enantiomers of solketal increased from $R = 1.43$ to 4.39. Further, the diastereomeric 1,3-dioxolane compound (compound **5**) could be separated into its four diastereomers. Also, the enantiomers of 2,3-dichloro- and 2,3-dibromo-1-propanol could be separated after derivatization. On the other hand, glycidol and glycerol formal (compound **1**) gave only one peak, probably caused by decomposition of the compounds. Further, although the separation seemed feasible for solketal, determination of the optical purity of an (*S*)-(+)-solketal sample (e.e. = 99.1%) resulted in a lower value (e.e. = 98.6%). When a five times higher TFAA concentration and a two times longer derivatization time were used, an even lower e.e. value was found (69.8%). Hence solketal, and probably all homologous 1,3-dioxolane alcohols, are liable to acid racemization during derivatization with TFAA.

Evaluation

Some years ago we managed to determine the e.e. value of glycidyl butyrate in aqueous samples by adapting the TAGIT derivatization method to this compound and separating the diastereomers by HPLC on a reversed-phase column [10]. As shown here, the method has been optimized and proved to be suited for other glycidyl esters and glycidol. As always the same diastereomers were found by HPLC, derivatization proceeds as suggested in Fig. 1, as sup-

TABLE IV

CHROMATOGRAPHIC PARAMETERS ON A CAPILLARY GC COLUMN WITH A CHIRAL (2,3,6-TRI-O-METHYL-β-CYCLODEXTRIN) STATIONARY PHASE OF THE TRIFLUOROACETYL DERIVATIVES OF ENANTIOMERS OF GLYCIDOL, SOLKETAL, HOMOLOGOUS 1,3-DIOXOLANE ALCOHOLS AND SOME OTHER ALCOHOLS

Terminology and abbreviations as in Table I.

Compound	Temperature (°C)	Retention times		α	R
		t_1 (min)	t_2 (min)		
Glycidol	45	16.8		NR	NR
Solketal	100	7.66 (*R*)	8.15 (*S*)	1.090	4.39
Compound **1**	70	20.5		NR	NR
Compound **3**	90	20.1	20.7	1.032	1.53
Compound **4**	110	26.2	27.0	1.033	1.93
Compound **5**	100	18.4	19.0	[a]	[a]
		19.8	21.7		
Compound **6**	125	12.2	12.7	1.049	1.26
Compound **7**	125	19.8	20.8	1.060	1.38
Compound **8**	140	46.6	47.1	1.012	0.65
2,3-Dichloro-1-propanol	90	10.5	10.8	1.031	1.40
2,3-Dibromo-1-propanol	110	12.6	13.0	1.039	2.20
3-Bromo-2-methyl-1-propanol	60	31.2	32.1	1.029	1.10
2-Phenyl-1-propanol	90	31.0	31.6	1.022	1.16
2-Octanol	50	49.5	50.5	1.021	1.00

[a] Compound **5** consists of four diastereomers. Four peaks were obtained with resolutions of $R \geq 1.33$ and separation factors of $\alpha \geq 1.035$.

ported by product identification. Therefore, glycidol and all glycidyl esters can be determined with this method. Control experiments showed that no racemization or kinetic enrichment occurred. As separation of the diastereomers was much better than for solketal, it is estimated that the accuracy at high e.e. values is at least ±0.1%. Modification of the derivatization procedure permitted the determination of solketal and homologous 1,3-dioxolane compounds. Also for these cases it was checked that no detectable racemization or kinetic enrichment occurred. Owing to the lower resolution, the accuracy at high e.e. values of solketal appeared to be ±0.2%.

During the course of our investigations, novel possibilities arose as GC columns with chiral stationary phases became available. The generally adapted strategy for determining racemic alcohols, which is to convert them into esters to obtain good separation characteristics, did not work here as the compounds either decomposed (glycidol) or racemized (solketal). Direct analysis without derivatization appeared to be possible, the γ-CD column being better suited for this than the β-CD column. The high resolution achieved resulted in high accuracies, as illustrated with experiments for solketal at high e.e. values (99.52 ± 0.07%). Despite its convenience, wide range of application and accuracy, the GC method has the disadvantages that the columns are expensive and dedicated to this particular purpose and glycidyl esters from hexanoate on could not be determined. Therefore, the HPLC method is still viable.

ACKNOWLEDGEMENTS

The authors thank Erik Keegstra for the synthesis of the homologous 1,3-dioxolane alcohols, Ir. A. Sinnema (Department of Organic Chemistry, Delft University of Technology) for providing ^{1}H NMR data and Andeno, Venlo, for the gift of racemic and (*R*)-(−)-glycidyl butyrate.

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