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Short communication

Analytical separation of the four stereoisomers of isopropyl 2,3-epoxybutanoate by gas chromatography

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Abstract

The isopropyl (2*R*,3*R*)- and (2*S*,3*S*)-*cis*-2,3-epoxybutanoates were prepared in three steps from L- and D-threonine, respectively. The racemic isopropyl *trans*-2,3-epoxybutanoate was obtained in two steps from *trans*-crotonic acid. The previous esters (four stereoisomers) could be easily differentiated by gas chromatography on a CP-Chirasil-Dex-CB capillary column.

Keywords: Enantiomer separation; Isopropyl 2,3-epoxybutanoate

1. Introduction

Enantiopure epoxides constitute an important family of chirons [1,2], useful to prepare various biologically active compounds and drugs [3–7]. Their high synthetic value results from the possibility to open the epoxide ring in a regional and stereocontrolled way [8,9].

In connection with the total synthesis of enzyme inhibitors, we required pure (2*R*,3*R*)- and (2*S*,3*S*)-*cis*-2,3-epoxybutanoic acids. These compounds were prepared from L- and D-threonine, respectively, and derivatized into the corresponding isopropyl esters which could be readily analyzed by gas chromatography (GC) [10,11].

Chiral chromatography recently developed as an essential and versatile tool for the analytical separation of enantiomers [12,13]. Such separations can be performed on cyclodextrin-based chiral stationary phases [14,15] via inclusion complexes; the method is routinely employed in high-resolution chromatography using capillary columns [16].

2. Experimental

2.1. Syntheses

(2*R*,3*R*)-*cis*-2,3-epoxybutanoic acid: the nitrous acid deamination of L-threonine (Fluka, ≥99.5%) in the presence of potassium bromide to give (2*S*,3*R*)-2-bromo-3-hydroxybutanoic acid **1a** was performed according to Refs. [17,18,21]. The sodium salt **2a** of (2*R*,3*R*)-2,3-epoxybutanoic acid was prepared by reaction of **1a** with aqueous NaOH (2 equiv.) at

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−5°C (1 h at −5°C, then 18 h at 20°C and freeze drying), according to a modified procedure from Refs. [19–22].

(2*S*,3*S*)-*cis*-2,3-epoxybutanoic acid: the sodium salt **2b** was prepared as above, starting from D-threonine (Fluka, allo free, ≥99%).

Isopropyl (2*R*,3*R*)-*cis*-2,3-epoxybutanoate: a mixture of crude **2a** (200 mg, 0.88 mmol) and 2-iodopropane (0.88 ml, 10 equiv.) in dry dimethylformamide (DMF, 2 ml) was stirred under argon atmosphere, at 20°C for 4 days, then poured into ice-cold water (10 ml) and extracted with ethyl acetate (3×15 ml). The organic layers were washed with brine (3×20 ml), dried and concentrated to furnish the ester **3a** as a pale yellow oil: yield=117 mg (92%); $[\alpha]_D^{20}=+13.7°$ (±0.2) (*c*=1.00; $CHCl_3$); IR (film) ν=2989, 2932, 1734 (C=O), 1457, 1261 cm^{-1}; ^1H NMR (C^2HCl_3+Me_4Si (TMS); 300 mHz) δ=1.28 and 1.30 (two d, 6H, *J*=6.3 Hz, $CHMe_2$), 1.39 (d, 3H, *J*=5.3 Hz, CHMe), 3.28 (dq, 1H, *J*=5.3 and 4.5 Hz, CH-3), 3.48 (d, 1H, *J*=4.5 Hz, CH-2), 5.14 (sept, 1H, *J*=6.3 Hz, $CHMe_2$); ^{13}C NMR (C^2HCl_3+TMS; 75 mHz) ppm=12.77, 21.69, 53.07, 53.27, 69.16, 167.73; MS(EI) *m*/*e*=145 (M+1, 2%), 102 (100%); Anal. calcd. for $C_7H_{12}O_3$: C; 58.32; H, 8.40; Found: C, 58.25; H, 8.52%.

Isopropyl (2*S*,3*S*)-*cis*-2,3-epoxybutanoate: the ester **3b** was prepared as above, starting from the salt **2b**: $[\alpha]_D^{20}=-13.7°$ (±0.2) (*c*=1.015; $CHCl_3$); Anal. calcd. for $C_7H_{12}O_3$: C, 58.32; H, 8.40; Found: C, 58.56; H, 8.47%.

(2*R*,3*S*)/(2*S*,3*R*)-*trans*-2,3-epoxybutanoic acids: the potassium salt of *trans*-2,3-epoxybutanoic acid (**2c**/**2d**: racemic mixture) was prepared by oxidation of *trans*-crotonic acid with oxone according to Ref. [23].

Isopropyl (2*R*,3*S*)/(2*S*,3*R*)-*trans*-2,3-epoxybutanoates: the procedure described for the preparation of **3a** was applied to furnish the racemic mixture **3c**/**3d**, as a pale yellow oil: yield=77%; ^1H NMR (C^2HCl_3+TMS; 200 mHz) δ=1.25 (d, 6H, *J*=6.5 Hz, $CHMe_2$), 1.42 (d, 3H, *J*=5.15 Hz, CHMe), 3.15 (d, 1H, *J*=2.2 Hz, CH-2), 3.22 (dq, 1H, *J*=5.15 and 2.2 Hz, CH-3), 5.10 (sept, 1H, *J*=6.5 Hz, CHMe).

2.2. Gas chromatography analyses

2.2.1. Equipment

We used a Carlo Erba Instruments GC 8000 chromatograph equipped with a flame ionisation detection (FID) system and coupled to a Merck–Hitachi D-2000 integrator for the non-chiral analyses, and a Carlo Erba Instruments HRGC 5300 chromatograph equipped with FID and coupled to a Merck–Hitachi D-2500 integrator for the chiral analyses. This equipment was supplied by Interscience (Parc Scientifique, Louvain-la-Neuve, Belgium).

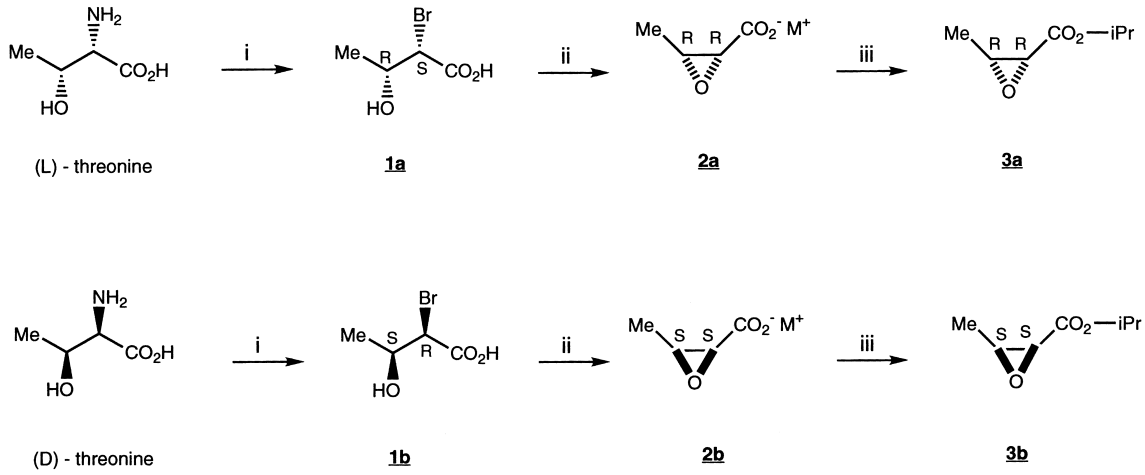

Fig. 1. Preparation of the *cis*-isomers **3a** and **3b**. Reagents and conditions: (i) $NaNO_2$, KBr, 2.5 *M* H_2SO_4, −3°C; (ii) NaOH aq., 0°C; (iii) iPr-I, DMF, 20°C.

2.2.2. Columns

We used an OPTIMA-5 capillary column from Machery–Nagel (30 m×0.25 mm; film thickness of 0.25 μm), supplied by Filter Service (Eupen, Belgium), for the non-chiral analyses, and a CP-Chirasil-Dex-CB [24] capillary column from Chrompack (25 m×0.25 mm; film thickness of 0.25 μm), supplied by Chrompack (Antwerp, Belgium), for the chiral analyses.

2.2.3. Carrier gas

The carrier gas was helium (purity: 99.999%; inlet pressure: 74 kPa (non-chiral chromatography) and 90 kPa (chiral chromatography).

2.2.4. Temperature

The analyses were performed in the isothermal mode at 80°C or 100°C (injector temperature= 220°C).

2.2.5. Sample preparation

The esters **3** were dissolved in acetone: concentration=1%; injection volume: 3 μl; split ratio: 1/100.

3. Results and discussion

The tungstate-catalyzed oxidation of *cis*-crotonic acid with H_2O_2 gave the *cis*-epoxide (**2a**+**2b**) as a racemic mixture [25], the resolution of which is not described in the literature. On the other hand, enantiopure *cis* epoxides **2a** and **2b** could be obtained, in a two-step sequence, starting from L- and D-threonine, respectively (Fig. 1) [21,22]. Reaction of threonine with nitrous acid, at low temperature, in the presence of potassium bromide gave the α-bromoacid **1a** or **1b**. This intermediate was readily cyclized into **2a** or **2b** using two equivalents of aqueous sodium hydroxide. Direct alkylation of the crude salt **2a** or **2b** was selected as non-epimerizing derivatization technique to produce volatile compounds that could be analyzed by high-resolution GC. We first prepared the methyl- and ethyl esters by reaction of **2a** or **2b** with methyl- and ethyl iodide, respectively. The recovered yields of esters were moderate due to their partial evaporation during the work-up. We then considered the isobutyl ester but, the isobutyl bromide was poorly reactive towards the carboxylate **2a** or **2b**, even in the presence of sodium iodide or a crown-ether as catalyst. We found that the isopropyl ester **3a** or **3b** was the best candidate to fulfil our purpose. Thus, reaction of **2a** or **2b** with an excess of 2-iodopropane, in DMF at room temperature for 4 days, quantitatively furnished the ester **3a** or **3b** (Fig. 1). Both esters were characterized by the same optical rotation, but with opposite signs. Their ^{1}H NMR spectrum showed a doublet and a doublet of quadruplet at 3.48 δ and 3.28 δ respectively, attributed to the H-2 and H-3 protons; their *cis*-stereochemical relationship was confirmed by the coupling constant value of 4.5 Hz. The two methyl groups of the isopropyl residue appeared non-equivalent in this sterically hindered configuration (two doublets near 1.3 δ). The ^{13}C NMR values we recorded were in agreement with a related previous report on glycidic derivatives (C-2 and C-3 near 53 ppm) [26].

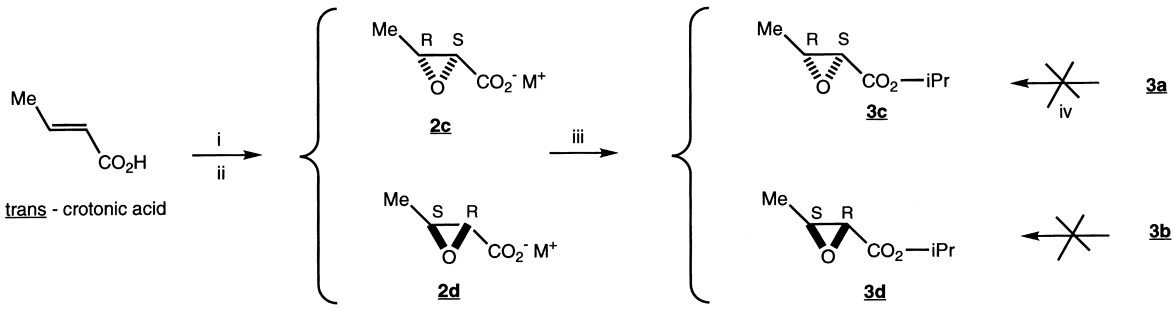

Fig. 2. Preparation of the *trans*-isomers **3c**/**3d**. Reagents and conditions: (i) oxone, Na_2EDTA ($4\cdot10^{-4}$ *M*), acetone, $NaHCO_3$ aq., 20°C; then acidic work-up; (ii) KOH, EtOH; (iii) iPr-I, DMF, 20°C; (iv) iPrOH, KOH, Δ.

The enantiopurity of the final epoxide **2a** or **2b** mainly depends on the stereoselectivity of the first step, i.e. the deamination, for which an intramolecularly assisted-mechanism has been proposed [27]: a partial stereochemical loss would result from some competition with the non-assisted nucleophilic substitution. Accordingly, the *cis*-epoxide **2a** or **2b** formed in the second step could be contaminated with the *trans*-epoxide **2c** or **2d** respectively. These reference compounds were thus prepared (Fig. 2).

Racemic *trans*-epoxide (**2c**/**2d**) could be obtained by oxidation of *trans*-crotonic acid [23,25,28,29]; the subsequent resolution with brucine, quinine or 1-α-naphthyl ethyl amine afforded the separated **2c** and **2d** enantiomers [30–32]. The asymmetric Sharpless epoxidation of *trans*-crotyl alcohol followed by $RuCl_3$–$NaIO_4$ oxidation also furnished the enantiomerically pure epoxides **2c** and **2d** [33]. At last, the microbial enantioselective reduction of α-chloroacetoacetate followed by base-catalyzed epoxidation conducted to **2c** and **2d** [34,35]. Nevertheless, for convenience reasons, we prepared the racemic mixture (**2c**/**2d**) according to the procedure of Carey and Ward [23]. The subsequent esterification with 2-iodopropane was conducted as previously described for the related *cis*-isomer (Fig. 2). The *trans*-epoxy-ester (**3c**/**3d**) is characterized in ^1H NMR spectroscopy by a doublet and a doublet of quadruplet at 3.15 δ and 3.22 δ, due respectively to the H-2 and H-3 protons. Their coupling constant value of 2.2 Hz is typical of the *trans*-stereochemical relationship.

The esters **3a** (one enantiomer), **3b** (one enantiomer), and **3c**/**3d** (racemic mixture) were analyzed by capillary GC with a non-chiral column (OPTIMA-5) and a chiral column (CP-Chirasil Dex-CB) [24]. The results are summarized in Table 1. The *cis*- and *trans*-isomers were easily differentiated, and the enantiomeric pairs gave well separated peaks on the chiral column. The Fig. 3 illustrates the analytical separation of a crude epoxybutanoates mixture prepared from racemic **3c**/**3d** and enantiopure **3a** and **3b**.

The *cis*-epoxides (Figs. 1 and 4) prepared from enantiopure threonines appeared optically pure and non-contaminated with the *trans*-isomers (limit of detection 0.1%). However, for one batch of **3a** corresponding to a synthesis during which the temperature control of the first step was not perfectly sure, we observed 2.1% of contamination with the *trans*-isomer **3c**. We further controlled that the *cis*-epoxy-esters **3a** and **3b** were configurationally stable when heated in isopropanol solution in the presence of a base (80°C, KOH, 24 h; see Fig. 2).

4. Conclusion

In this short communication, we disclose a simple and useful method for controlling the enantiomeric and diastereoisomeric purity of epoxy-acid derivatives transformed into volatile isopropyl esters. The GC analyses could be performed directly on the crude derivatization mixtures (containing DMF and i-PrI). Due to the absence of a good chromophoric group on the substrate and the presence of an epoxide ring sensitive to nucleophilic solvents (H_2O,

Table 1
GC analysis of the epoxybutanoates **3**

Compounds	Optima-5 column		CP-Chirasil-Dex-CB column			
	100°C t_R	80°C t_R	100°C t_R	80°C t_R	α	R_s
cis-Epoxides	6.4	10.5				
3a (2*R*,3*R*)			6.0	13.9	1.19	9.2
3b (2*S*,3*S*)			5.4	11.6		
trans-Epoxides		9.9				
3c (2*S*,3*R*)				9.9	1.06	3.0
3d (2*R*,3*S*)				10.5		

t_R=retention time in minutes; analysis conditions: see Section 2.

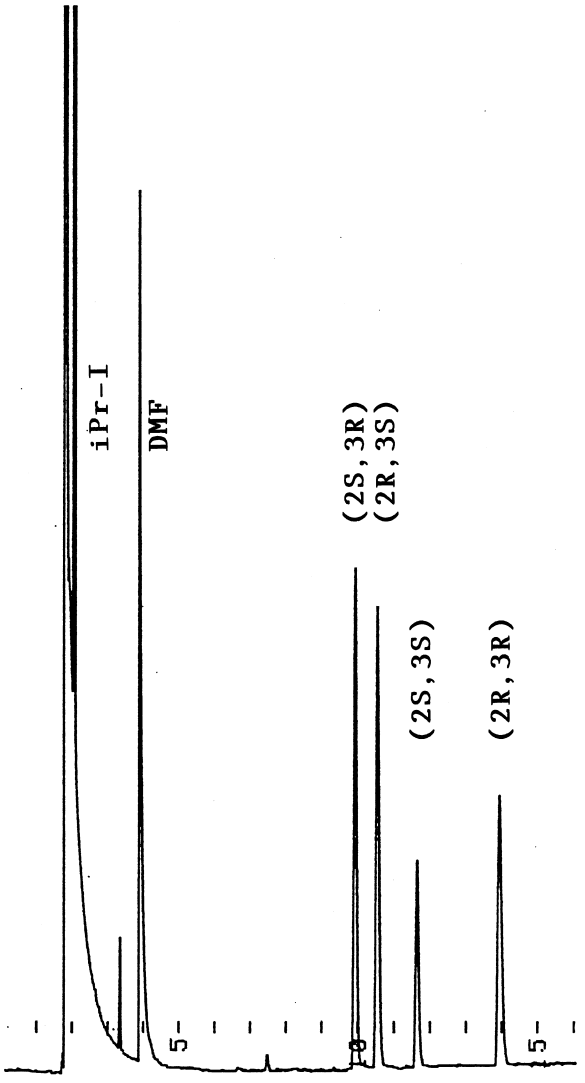

Fig. 3. Gas chromatography of a mixture of the four stereoisomers **3** on the chiral column.

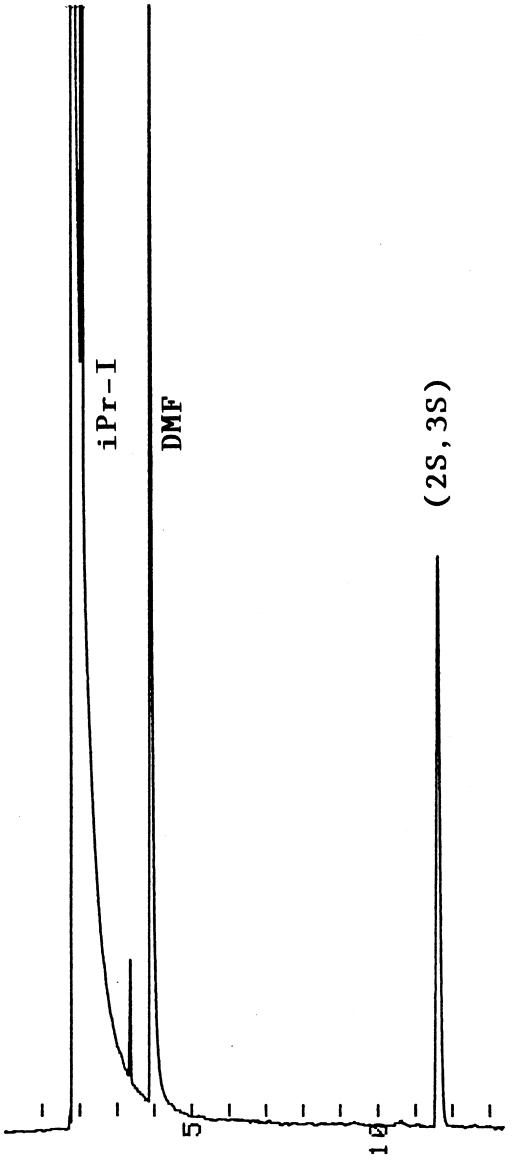

Fig. 4. Gas chromatography of enantiopure **3b** on the chiral column.

MeOH), the GC analysis was, in our particular case, largely superior to the HPLC technique.

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