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1-Phenylethyl isocyanate is a powerful reagent for the chiral analysis of secondary alcohols and hydroxy fatty acids with remote stereogenic centres

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Abstract

1-Phenylethyl isocyanate (1-PEIC), a chiral derivatisation reagent for the resolution of secondary alcohols is a powerful tool to determine the configuration and enantiomeric excess of medium- to long-chain secondary alcohols by capillary gas chromatography. The separation of 1-phenylethylcarbamates (1-PECs) of secondary alcohols was systematically evaluated depending on the position of the stereogenic centre in the molecule, namely in alkanols (C_{15}–C_{18}), alkenols (C_{15}–C_{18}) and hydroxy fatty acids (C_{14}–C_{18}). The successful separation of the diastereomeric carbamates of (±)-heptadecan-7-ol or (±)-12-hydroxyoctadecanoic acid methyl ester by gas liquid chromatography demonstrates the unique separation power for 1-PECs for analytes with remote stereogenic centres. Saturated derivatives showed consistently higher resolution factors than the corresponding unsaturated derivatives.

Keywords: 1-Phenylethyl isocyanate (1-PEIC); Derivatisation; Gas liquid chromatography; Diastereomeric separation; Hydroxy fatty acids; Oxylipins

1. Introduction

Chirality is an intrinsic feature of many compounds, in particular of natural products, and is often indispensable for their function. Accordingly, the analysis of the enantiomeric excess (ee) of bioactive compounds is of great interest for many disciplines. The configuration and the enantiomeric excess of compounds can be either investigated directly by interaction with chiral stationary phases, or indirectly after transformation into diastereomers by derivatisation with a chiral reagent. The separation of enantiomers becomes especially challenging, if the stereogenic centre is remote from functional groups, as is often the case in bioactive hydroxy fatty acids [1,2], pheromones [3,4], or long-chain secondary alcohols from leaf surfaces [5]. In these cases the difference between the substituents attached to the stereogenic centre is low and a direct separation of the enantiomers becomes almost impossible. Solutions to determine remote stereogenic centres have been reported for NMR approaches with chiral shift reagents, chiral solvents or after derivatisation with chiral reagents [6,7]. However, a major disadvantage common to these methods is the necessity to purify the analytes and the rather large amount needed for NMR. More sensitive are direct or indirect HPLC methods that have been developed for the assignment of the stereochemistry of broad range of hydroxy fatty acids [8]. For example, the derivatisation of 15-hydroxyeicosatetraenoate with dehydroabietyl isocyanate afforded carbamates that displayed baseline resolution on silica gel [9]. Successful separations were also reported for *Mosher* esters of 9- or 12-hydroxyoctadecadienoates [10]. Due the ease of derivatization and their versatile chiral analyses by NMR, gas chromatography (GC) and high-performance liquid chromatography (HPLC), the formation of *Mosher* esters belongs to the most popular methods [11,12]. Chiral stationary phases, e.g. (*R*)-(−)-*N*-3,5-dinitrobenzoylphenylglycine (DNBPG) or modified celluloses (Chiracel) allowed direct separation of the enantiomers of 9-hydroxy-10*E*,12*Z*,15*Z*-octadecatrienoic acid and 13-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (9- and 13-HOTE) [13,14], and 8-hydroxy-5*Z*,9*E*,11*Z*,14*Z*-eicosatrienoic acid, 12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatrienoic acid and 15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatrienoic acid (8-, 12- and 15-HETE) [15,16] without the need for a preceding derivatisation. However, two critical factors limit the use of these methods. First, the complexity of many natural samples and, second, insufficient separation of enantiomeric analytes with

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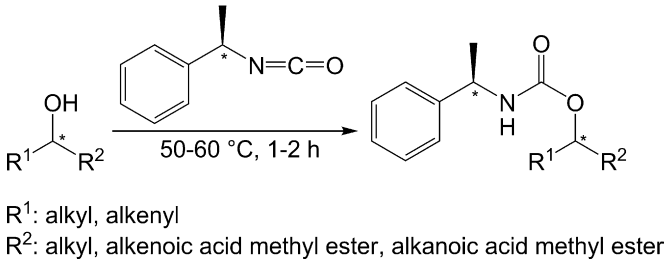

Fig. 1. Derivatisation of alcohols and hydroxy fatty acids with (*R*)-1-phenylethyl isocyanate ((*R*)-1-PEIC).

stereogenic centres remote from other functional groups (e.g. double bonds).

The problem of sample complexity can be addressed by high-resolution gas chromatography, but insufficient separation can only be overcome by powerful derivatisation reagents or the choice of appropriate chiral stationary phases. For example, the commonly employed *Mosher* esters failed to separate the enantiomers of 17-hydroxy-octadecanoic acid, but their corresponding 1-phenyethyl carbamates (1-PECs) allowed separation and assignment of the stereochemistry of the 17-hydroxy group [17,18]. The derivatisation with phenylethyl isocyanate (1-PEIC) was introduced in the late 1960s and has been used since then for the chiral analysis of secondary alcohols, in pheromones [19], lactones after ring-opening to hydroxycarboxamides (δ- and γ- lactones with chain lengths up to C_{12}) [20,21], terpenoids, for example linalool [22,23], in flavour and fragrance analysis and to determine the configuration and ee of biologically active hydroxy fatty acids [24,25], for example 5-hydroxydecanoate (Fig. 1).

The method was also applied to separate several long-chain 2-alkanols (C_{12}–C_{24}) [26] and hydroxy fatty acids [18,26], for example, methyl 19-hydroxyeicosanoate and methyl 21-hydroxydocosanoate, but most of the reported examples refer to stereogenic centres close to the terminus of the molecules.

The facile separation of the diastereomeric carbamates of 17-hydroxyoctadecanoic acid methyl ester [17] and some related examples prompted us to investigate whether or not the derivatisation with 1-PEIC can be more generally applied to address the configuration and ee of secondary alcohols in central segments of long-chain hydroxy compounds. Here we demonstrate the separation of the enantiomers of 1-phenylethyl carbamates obtained from a wide range of acyclic aliphatic hydroxy compounds by gas chromatography on the stationary phase EC-5 (5% phenyl, 95% methylpolysiloxane). Successfully resolved were 1-PECs of saturated alcohols, alkenols and hydroxy fatty acids with stereogenic centres remote from functional groups or the terminus of the molecule.

2. Experimental

2.1. Instrumentation

GC analysis was performed with a Trace GC (Thermo Scientific, USA) connected to a Trace MS detector (Thermo Scientific). Compounds were separated on different capillary columns: EC-5 (15 m × 0.25 mm I.D., 0.25 μm film thickness, Alltech, Munich, Germany), Zebron ZB-1 (30 m × 0.25 mm I.D., 0.25 μm film thickness, Phenomenex, Aschaffenburg, Germany), DB-WAX (30 m × 0.25 mm I.D., 0.25 μm film thickness, J&W, Agilent Technologies, Waldbronn, Germany) and RTX-200 (30 m × 0.25 mm I.D., 0.25 μm film thickness, Restek, Bad Homburg, Germany). Helium at 1.2 or 3 ml min^{-1} served as carrier gas. Samples were injected in the splitless mode. Injection port, transfer line and ion source were kept at 270, 280 and 200 °C, respectively. Electron ionisation (EI) spectra (70 eV) were recorded from *m/z* 41 to 550. Separations with hydrogen as carrier gas were achieved on a GC8060 (Fisons, GB) connected to a MD800 MS detector (Fisons, GB) equipped with an EC-5 capillary column (15 m × 0.25 mm I.D., 0.25 μm film thickness, Alltech, Munich, Germany) or a DB5 capillary column (30 m × 0.25 mm I.D., 1 μm film thickness, J&W, Agilent Technologies, Waldbronn, Germany). The GC was operated at a constant pressure of 1 psi hydrogen using the same temperature program as for helium.

2.2. Chemicals

(*R*)- and (*S*)-1-PEIC were purchased from Fluka. The optical purity of the reagent was between 97 and ≥99.5% (the highest commercially available optical purity, Fluka Chiraselect). Methyl 5-, 7-, and 10-hydroxyoctadecanoate were obtained by reduction with $NaBH_4$ from commercially available methyl 5-, 7-, or 10-oxooctadecanoate (Sigma–Aldrich). Ricinoleic acid methyl ester was purchased from Sigma–Aldrich. 9-HODE (9*S*)-9-Hydroxy-(10*E*, 12*Z*)-octadecadienoic acid, 13-HODE (13*S*)-13-hydroxy-(9*Z*,11*E*)-octadecadienoic acid were obtained from α-linolenic acid by enzymatic peroxidation with soybean lipoxygenase (ca. 99% ee) [27]. Methyl 3-oxooctadecanoate was prepared according to literature [28] followed by $NaBH_4$ reduction. Solvents and other chemicals indicated in the procedures were purchased from Aldrich, Fluka or Alfa Aesar, and were used without further purification.

2.3. General procedure for the synthesis of alk-1-enols

A solution of alkenylmagnesium bromide, prepared from an alk-1-enyl bromide (4 mmol) and magnesium turnings (6 mmol) in Et_2O (5 ml) was transferred via a cannula into a stirred suspension of cuprous iodide (0.28 mmol, 7 mol%) in THF (20 ml) at −40 °C under argon. After 10 min the racemic or chiral terminal oxirane (3 mmol) in THF (5 ml) was added dropwise. Stirring was continued for 4 h at −40 °C and then at room temperature (RT). After hydrolysis with saturated NH_4Cl (aqueous solution, 10 ml), the products were extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The crude product was dissolved in pentane and kept at low temperature (−20 to −80 °C) for crystallisation. The alk-1-enols were obtained as colourless crystals in 70–90% yield. Short-chain alk-1-enols (<C12) were purified by flash column chromatography using hexane:Et_2O (1:1; v/v) for elution.