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Stereochemical characterization of fluorinated 2-(phenanthren-1-yl)propionic acids by enantioselective high performance liquid chromatography analysis and electronic circular dichroism detection

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ABSTRACT

Enantioselective high performance liquid chromatography (HPLC) coupled with a detection system based on the simultaneous measurement of UV absorption and electronic circular dichroism (ECD) allows a complete stereochemical characterization of chiral compounds, once the relationship between sign of the chiroptical properties and absolute configuration is determined. In the present communication, the development of enantioselective HPLC methods for the resolution of a series of fluorinated 2-phenanthrenylpropionic acids (**1**–**6**) is reported. Different chiral stationary phases (CSPs) were tested: Chiralcel® OJ, Chiralcel® OD, Chiralpak® AD, (*S*,*S*)-Whelk-O® 1, Chirobiotic™ T and α_1-acid glycoprotein (AGP). The results allow the application of the methods to a reliable determination of the enantiomeric excess for all the examined compounds; the highest enantioselectivity values were obtained with the Hibar® [(*S*,*S*)-Whelk-O® 1] column for some of the examined compounds. In the case of *rac*-2-(6-fluorophenanthren-1-yl)propionic acid (**1**), the relationship between circular dichroism and absolute configuration of the enantiomeric fractions was determined by ECD analysis and time-dependent density functional theory (TD-DFT) calculations. The experimental ECD spectrum of the second-eluted fraction of **1** on the Hibar® [(*S*,*S*)-Whelk-O® 1] column was found to be in excellent agreement with the theoretical ECD spectrum of (*S*)-**1**; therefore, the absolute configuration of the first- and second-eluted enantiomers on the (*S*,*S*)-Whelk-O® 1 CSP was assessed as (*R*) and (*S*), respectively, and the elution orders of the enantiomeric forms of **1** were determined on all the different CSPs.

1. Introduction

Fluorinated profens have received particular consideration in reference to their biological activity as successful non-steroidal anti-inflammatory drugs (NSAIDs), especially in connection with the presence of fluorine in the molecule. In fact, once placed in a specific position of a bioactive molecule, fluorine can substantially affect its chemical stability [1–6], thus slackening the related metabolic processes. Moreover, since the C–F bond is stronger than the C–H bond and exhibits reverse polarity, replacement of the α-hydrogen with the quasi-isosteric fluorine conveys a higher configurational stability to the chiral center of profens [7], thus allowing the drug pharmacodynamics, as well as the stereochemical matching with the biological target, to be investigated.

Since the biological activity of 2-(phenanthren-1-yl)propionic acid as a NSAID was reported to be similar to that of fenbufen [8], a number of nucleus and/or side-chain fluorinated 2-phenantrylpropionic acids were prepared [9], in order to assess the effect of fluorine on the structure/activity relationship with respect to its position and to the configuration of the chiral carbon. Nevertheless, a reliable study on the relationship between stereochemistry and biological activity requires a full stereochemical characterization of the compounds under investigation.

In this article, the development of enantioselective high performance liquid chromatography (HPLC) methods for the resolution of a series of 2-(fluorophenanthren-1-yl)propionic acids (**1**–**6**, Fig. 1) is reported; these methods may be applied for the determination of the enantiomeric excess (e.e.). For one of these compounds, 2-(6-fluorophenanthren-1-yl)-propionic acid (**1**), the enantioselective method has been scaled up to allow the collection of the enantiomeric fractions, before their stereochemical characterization. The e.e. value was determined through the same chromatographic

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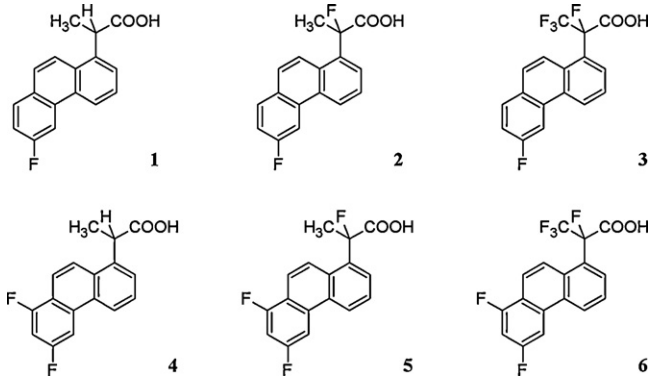

Fig. 1. 2-(Fluorophenanthren-1-yl)propionic acids.

assay, and the absolute configuration was assessed by electronic circular dichroism (ECD) spectroscopy and time-dependent density functional theory (TD-DFT) computations.

2. Experimental

2.1. Materials

Compounds **1**–**6** were prepared as previously reported [9]. *n*-Hexane, acetonitrile, methanol, ethanol, 1-propanol, 2-propanol, glacial acetic acid, formic acid, triethanolamine and triethylamine were purchased from Sigma–Aldrich (Milan, Italy). All solvents used to prepare solutions and mobile phases were HPLC or analytical grade. K_2HPO_4 and KH_2PO_4 powders were purchased from Carlo Erba Reagenti (Milan, Italy). Water was doubly distilled and buffers were filtered through a 0.22 μm membrane filter.

2.2. HPLC analysis

2.2.1. Instrumentation

A Varian (Palo Alto, CA, USA) Mod. 5000 HPLC system with a HP 1040A diode array detector (Hewlett Packard, Waldbronn, Germany) was used for the development of enantioselective methods and the collection of enantiomeric fractions. The e.e. determination with simultaneous monitoring of UV and ECD signals of compound **1** were carried out using a Jasco (Tokyo, Japan) HPLC system consisting of a Jasco PU-980 pump, a Jasco MD 910 multiwavelength detector and a CD-995 chiral detector, equipped with a 25 mm pathlength HPLC flow cell. Samples were injected by a 20 μL loop in both instruments.

The following HPLC columns were used, having different chiral stationary phases (CSPs): Chiralcel® OJ (250 × 4.6 mm I.D., 10 μm) and Chiralpak® AD (250 × 4.6 mm I.D., 10 μm), purchased from Daicel, Chiral Technologies Europe, Illkirch, France; Chiralcel® OD (250 × 4.6 mm I.D., 10 μm; Daicel, Millinckrodt Baker B.V., Deventer, Holland); Hibar® pre-packed column RT (250 × 4.6 mm I.D., 5 μm, customized packing (*S*,*S*)-Whelk-O® 1; Merck KGaA, Darmstadt, Germany); Chirobiotic™ T (250 × 4.6 mm I.D., 5 μm; Astec, Whippany, NJ, USA), and Chiral-AGP (100 × 4.0 mm I.D., 5 μm; ChromTech AB, Sollentuna, Sweden).

2.2.2. Chromatographic conditions

Stock solutions of compounds **1**–**6** (1.0 mg mL^{-1}) in 2-propanol and in methanol were prepared for direct-phase and reversed-phase chromatography, respectively. Stock solutions were further diluted in the same solvents to a concentration range between 0.02 and 0.1 mg mL^{-1}.

Mobile phases used in direct-phase chromatography were prepared with hexane and 2-propanol, adding formic acid or acetic acid to improve the chromatographic resolution. In particular, hexane/2-propanol/acetic acid mobile phases were used with Chiralcel® OJ (79:20:1; 79.5:20:0.5; 69:30:1; 89.5:10:0.5; 80:20:0, v/v/v), and Hibar® [(*S*,*S*)-Whelk-O® 1] columns (80:20:0; 79.5:20:0.5; 90:10:0; 89.5:10:0.5; 94.5:5:0.5, v/v/v), while hexane/2-propanol/formic acid mobile phases were used with Chiralcel® OD (69.5:30:0.5; 79.5:20:0.5; 89.5:10:0.5, v/v/v) and Chiralpak® AD (70:30:0; 69.5:30:0.5, v/v/v) columns.

The Chiralcel® OD column was also successfully used to separate and collect the single enantiomers of compound **1** (mobile phase, 79:5:20:0.5, v/v/v hexane/2-propanol/formic acid; flow 1 mL min^{-1}, λ: 250 nm). The e.e. of each fraction of **1** was determined by using the (*S*,*S*)-Whelk-O® 1 CSP (mobile phase, 79.5:20:0.5 v/v/v hexane/2-propanol/acetic acid; flow 1 mL min^{-1}, λ: 295 nm). The enantiomeric fractions were dried under nitrogen, reconstituted with 2-propanol and analyzed by UV spectroscopy to determine their concentration.

In reversed-phase chromatography, solutions of triethylamine (TEA) acetate buffer at different pH values mixed with methanol or acetonitrile and phosphate buffer/1-propanol (PB) were used with Chirobiotic™ and Chiral-AGP columns, respectively.

Triethylamine (TEA) acetate buffer was prepared adding acetic acid to aqueous triethylamine to adjust the pH value. The following mobile phases were used: TEA acetate (20 mM, pH 5, 6 and 7)/methanol 90:10, v/v; TEA acetate (20 mM, pH 6)/methanol (75:25; 80:20; 95:5, v/v); TEA acetate (20 mM, pH 6)/acetonitrile (80:20; 90:10; 95:5, v/v). Replacement of triethylamine by triethanolamine did not show any significant difference. Phosphate buffer (PB, 0.1 M, pH 6) was obtained by adding a 0.1 M aq. KH_2PO_4 (pH ∼ 4) to 0.1 M aq. K_2HPO_4 (pH ∼ 9). Mobile phases consisted in PB/1-propanol mixtures (90:10; 95:5, v/v). All mobile phases were degassed by sonication. Flow rate was maintained between 0.6 and 1.5 mL min^{-1}.

The enantioselectivity (α) was calculated as $\alpha = k'_2/k'_1$, where k'_2 and k'_1 are the capacity factors of the second- and first-eluted enantiomers, respectively. Capacity factors (k') are defined as $k' = t_r - t_0/t_0$, where t_r is the retention time of the analyte, and t_0 is the retention time of a non-retained solute. The enantiomeric excess was determined as e.e. = ([A] − [B])/([A] + [B]) × 100, where [A] and [B] are the peak areas of the most and less abundant enantiomers, respectively.

2.3. UV and ECD measurements

UV spectra of compounds **1**–**6** were carried out on a spectrophotometer Jasco V-520, in 2-propanol at room temperature, using 1 cm pathlength cells.

ECD spectra (370–210 nm spectral range) of the single enantiomeric fractions of **1** were recorded on a Jasco J-810 spectropolarimeter, in 2-propanol at room temperature, using a 1 cm pathlength cell. Concentrations were adjusted to keep the absorbance in the optimum photometric range. Spectra were recorded at 0.5 nm intervals using a 2 nm spectral bandwidth, a 20 nm min^{-1} scan rate and a 4 s time constant. The actual concentration of the fractions of **1** was determined by UV analysis.

2.4. Conformational analysis and ECD calculation

Molecular mechanics (MM) calculations were carried out for a preliminary conformational analysis of (*S*)-**1** (Fig. 2). The conformer distribution was determined at the MMFF94s [10] level using the Spartan'02 software [11], and the relative energies (ΔE_{MM}) with