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Note

Separation of the enantiomers of a triester of 2,2-difluorocitrate

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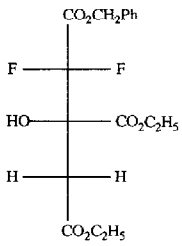
Racemic fluorocitrates have been used extensively in studies[1–4] related to the blocking of the citric acid cycle. They inhibit a number of enzymes such as aconitase[1] and succinic hydrogenase[2]. In the case of monofluorocitrate, only the (−)-enantiomer is biologically active, displaying sever CNS toxicity. Because of the lack of toxicity associated with the corresponding (+)-enantiomer of monofluorocitrate, it has been used as an NMR probe for the quantitative estimation of magnesium ions in a biological environment[6]. Although the fluorine atoms in 2,2-difluorocitric acid are not magnetically equivalent, and lead to two distinguishable signals in the fluorine NMR spectrum, difluoro citrate has not yet been used as an NMR probe. Separation of the enantiomers of the citric acid analogue **1** is not only of interest to afford knowledge on the biochemistry of these substances, but may also provide a useful precursor for a versatile NMR probe.

The enantiomers of 2,2-difluorocitrate have not been separated either by chemical or chromatographic methods. In this article, we describe the successful chromatographic separation of the antipodes of **1** and their characterisation by polarimetry and circular dichroism.



1

EXPERIMENTAL

Chemicals

n-Hexane (Rathburn) and propan-2-ol (BDH) were filtered through a Millipore Durapor 0.45-μm membrane filter and degassed with helium before use. Racemic **1** was prepared from the known diethyl difluorocitrate[7] and treatment with phenyldiazomethane.

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High-performance liquid chromatography (HPLC)

The HPLC system used to carry out the chromatographic separation of the enantiomers of **1** consisted of a Perkin-Elmer Series 4 liquid chromatograph, a Perkin-Elmer ISS-100 autoinjector and a Kratos Spectroflow 783 variable-wavelength absorbance detector operated at 210 nm. For the analytical separations the chiral column (250 × 4.9 mm I.D.) was of the Pirkle type (supplied by Hichrom) and was packed with (L)-N-(3,5-dinitrobenzoyl)leucine covalently bound to 3-amino-propyl silica (particle size 5 μm). The column was operated at 0°C. The best separation of the enantiomers was achieved using *n*-hexane–propan-2-ol as the mobile phase (97:3) at a flow-rate of 1 ml min^{-1}.

The preparation of about 40 mg of each of the two enantiomers was carried out using the same type of column packing as above. However, in this case the dimensions of the semi-preparative column were 250 × 8.0 mm I.D. Again, the same solvent ratio was used and the flow-rate was set at 2 ml min^{-1}.

Polarimetry and circular dichroism

In order to determine the enantiomeric composition of the separated enantiomers of **1**, their specific rotation $[\alpha]_D^T$ was measured using a Perkin-Elmer 241 polarimeter set at a wavelength of 589 nm (sodium D-line). Optical rotation was determined in a cell of 100 mm pathlength. Each of the enantiomers was dissolved in acetonitrile and equilibrated at 25°C before the specific rotation was measured. The concentrations of the (+)- and (−)-enantiomers were 9.6 and 9.2 mg ml^{-1}, respectively.

The circular dichroism (CD) spectra of the enantiomers were recorded from 350 to 225 nm on a Jasco J-600 spectropolarimeter, using a 10-mm pathlength cell thermostatted at 25°C. The (+)- and (−)-enantiomers were dissolved in acetonitrile, at concentrations of 0.96 and 0.92 mg ml^{-1}, respectively.

RESULTS AND DISCUSSION

The analytical separation of the enantiomers of **1** is shown in Fig. 1. The (−)-enantiomer elutes faster than the (+)-form. The retention times are 21.2 and 22.6

Fig. 1. HPLC separation of the enantiomers of **1**. For conditions, see Experimental.

min, respectively, and the corresponding separation factor, α, is 1.08.

To obtain about 40 mg of each of the two enantiomers a semi-preparative column was used. This enabled the injection of about 2-mg quantities of **1**. Fig. 2a shows a typical chromatogram obtained on this column. The rather peculiar shape of the peaks is due to overloading. Three fractions were collected. The first and third fractions gave the (−)- and (+)-enantiomer in 97 and 90% purity as determined by rechromatography on the analytical column using hexane–propan-2-ol (99:1) and a flow-rate of 1 ml min^{-1} (Fig. 2b and c). This purity was adequate for partial characterisation and for our research purposes.

The specific rotations $[\alpha]_D^{25}$ for fractions 1 and 3 were measured as −7.6 and +6.6. Taking into account the level of impurities of the (+)- and (−)-forms in either of the two fractions, the specific rotation of the pure enantiomers was calculated at about 8.1 with the appropriate sign.

The CD curves obtained from fractions 1 and 3 are shown in Fig. 3. As expected from the purity of the (−)- and (+)-enantiomers in the two fractions, one of these spectra is weaker than the other. The CD curves obtained in the wavelength range of 270–235 nm are due to the phenyl chromophore in **1**, which also gives rise to near-UV absorption maxima at 261, 257 and 252 nm with extinction coefficients of about 400 l mol^{-1} cm^{-1}. The CD band with a peak at 243 nm, which does not coincide with any

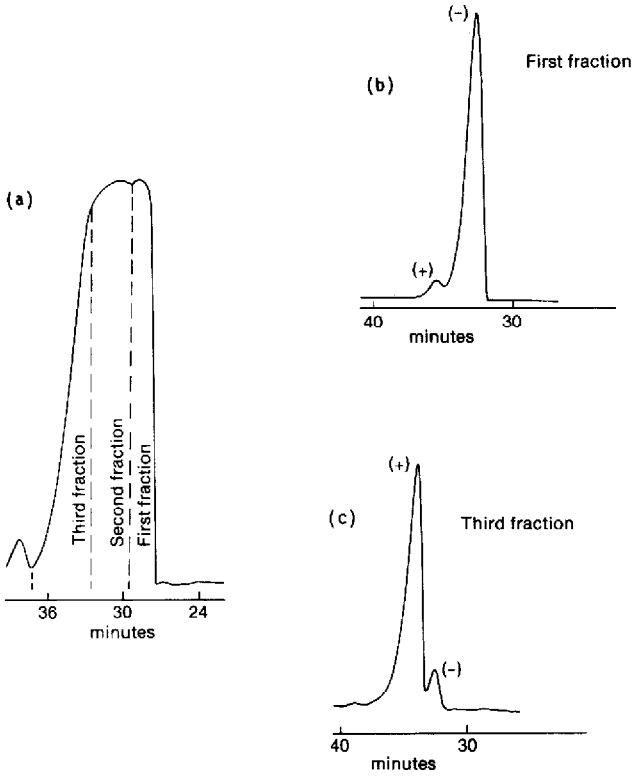

Fig. 2. Preparative HPLC of **1**. (a) Racemic mixture chromatographed on a semi-preparative column; (b) first fraction and (c) third fraction from (a) rechromatographed on an analytical column.

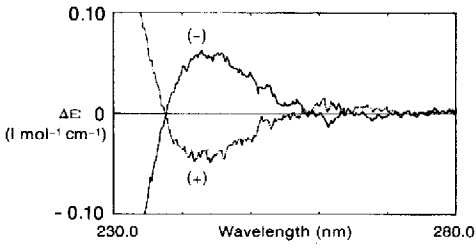

Fig. 3. CD spectra of the first and third fractions (see Fig. 2) denoted by (−) and (+), respectively. $[\alpha]_D^{25} = -7.6$ and $+6.6$.

of the UV absorption maxima, is probably due to the summation of two or more underlying positive and negative bands arising from the phenyl chromophore coupling with the two carbonyl chromophores of the ethyl ester groups in **1**.

In conclusion, the simple preparation procedure described in this note allows the preparation of milligram quantities of the (−)- and (+)-enantiomers of an ester of 2,2-difluorocitric acid in purity >90%. Larger quantities can be prepared using a larger preparative column and higher purity material can easily be obtained by rechromatography.

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