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Short Communication

Stereochemical analysis of a leukotriene related hydroxypentadecadiene using a chiral high-performance liquid chromatography column and diode array detection

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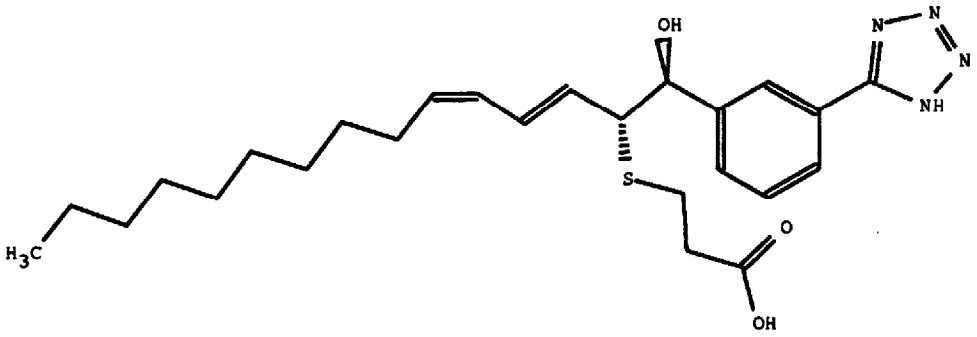
ABSTRACT

A high-performance liquid chromatographic separation of the chiral isomers of a leukotriene related hydroxypentadecadiene has been achieved using a chiral protein column which permits low levels of the undesired distomer to be measured in the eutomer. Diode array and proton magnetic resonance analytical methods were used to confirm their diene stereochemistry and that of a related impurity produced in trace amounts in the synthesis.

INTRODUCTION

The chiral hydroxypentadecadiene **Ia** is a leukotriene antagonist which is potentially useful in the treatment of asthma [1].



Ia. 5-(3-[2(R)-(carboxyethylthio)-1(S)-hydroxypentadeca-3(E),5(Z)-dienyl] phenyl-1H-tetrazole.

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With the current importance [2,3] of the chiral purity of new drug substances in mind we have developed a high-performance liquid chromatographic procedure which separates the enantiomeric forms of this *cis–trans* diene (**Ia,b**) and allows the pharmacologically undesirable (distomer) impurity (**Ib**) in the eutomer (**Ia**) to be monitored to less than 0.5%. The HPLC analysis also shows the presence of any of the analogous *trans–trans* diene isomer **II** which has the same 2(*R*),1(*S*) configuration as **Ia** and is the most likely geometrical impurity.

Early attempts to separate the underivatised chiral isomers of **Ia,b** using α-, β- or γ-cyclodextrin columns were unsuccessful. Chemical derivatisation with diazomethane to mask the acid function was also unhelpful and led only to a complex mixture of methylation products. A more successful derivatisation of **Ia,b** was performed by reaction of the carboxyl group with chiral α-methylbenzylamine [4] and separation of the resultant diastereomeric mixture with a non-chiral, unbonded silica column. The latter method, however, involves an overnight chemical reaction followed by a 40-min HPLC analysis time. In addition it does not offer an absolute method of determining low distomer impurity levels because of the uncertain chiral purity of commercial α-methylbenzylamine and the risk of epimer formation during derivatisation.

EXPERIMENTAL

High-performance liquid chromatography

The chromatographic system consisted of a Spectra-Physics SP 8800 pump and Spectra 200 detector (Spectra-Physics, Hemel Hempstead, U.K.). The eluent (acetonitrile–water (50:50) with 0.1% acetic acid) was pumped at 0.9 ml/min. The column was a chiral AGP (Chrom Tech, Norsborg, Sweden; dimensions 100 × 4 mm) and the analyte concentration was 100 μg/ml (2 μg injected on column).

Diode array detection

The diode array detector was a Hewlett-Packard Model 1040A (Hewlett-Packard, Wokingham, U.K.) consisting of 205 diodes together with a Hewlett-Packard series 9000/300 computing system.

Proton magnetic resonance spectra

The spectra were measured on a Bruker AM 300 spectrometer at 300 MHz using [$^2H_4$]methanol as solvent (Bruker Spectrospin, Coventry, U.K.).

RESULTS AND DISCUSSION

Experiments with the recently developed α_1-acid glycoprotein chiral column (Chiral AGP) [5] on the underivatised hydroxypentadecadienes were immediately successful. Using the conditions described above, a mixture containing 66.0% of the eutomer **Ia** and 30.8% of the distomer **Ib** was baseline resolved and clearly separated from the *trans–trans* diene chiral impurity (**II**, 3.2%). The total HPLC run time was less than 5 min.

The diene stereochemistries in **Ia,b** and **II** were clearly revealed by the vicinal alkene couplings in their high field (300 MHz) proton magnetic resonance spectra and by a diode array analysis of the three peaks marked in Fig. 1.

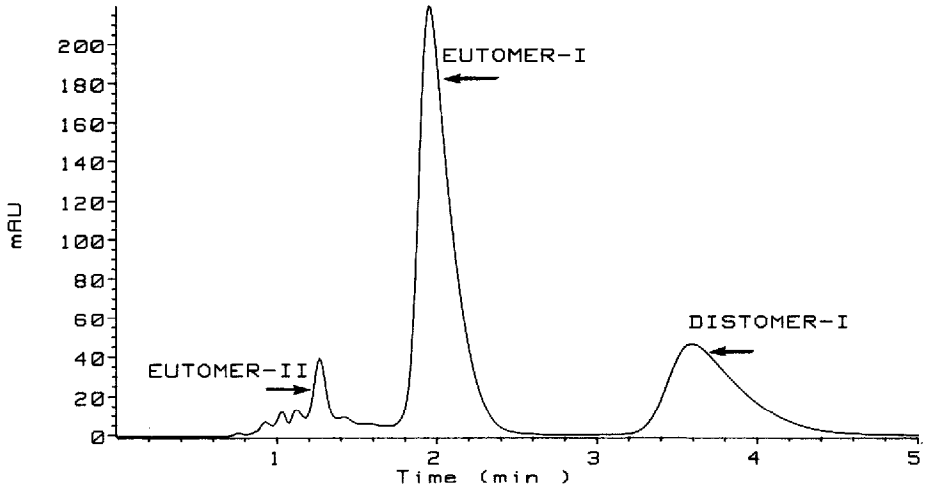

Fig. 1. HPLC separation of the eutomer and distomer of **I (Ia,b)** and of the related diene impurity **II** using Chiral AGP column. Eluent, acetonitrile–water (50:50) with 0.1% acetic acid.

The wavelength maxima for the two chiral isomers of **Ia,b** were, as expected, identical but the maximum for the diene stereoisomer **II** showed a small hypsochromic shift of 2 nm (Fig. 2). This shift agrees well with that reported for pairs of *cis–trans* and *trans–trans* diene systems [6].

Typical synthesised batches of the pure chiral hydroxypentadecadiene **Ia** were found to contain less than 1% of the distomer (**Ib**) and less than 1% of the *trans–trans* diene **II**.

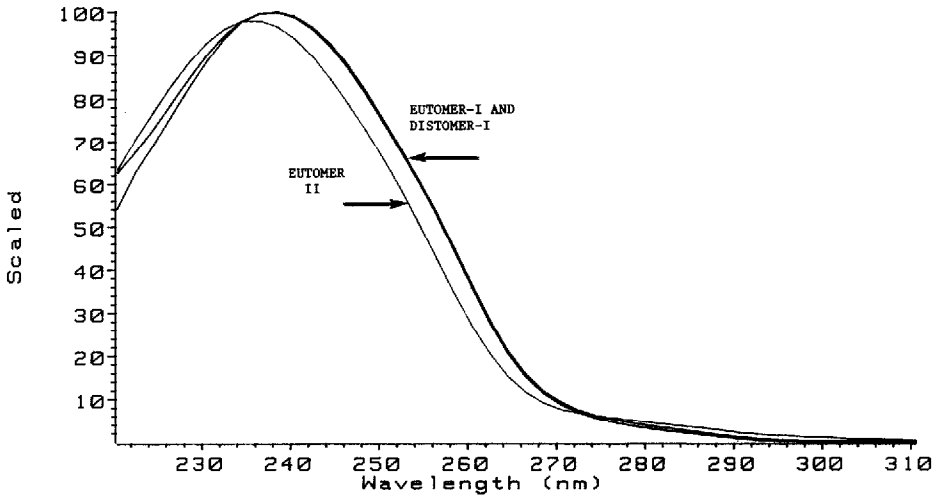

Fig. 2. Diode array analysis of the eutomer and distomer of **I (Ia,b)** and the related diene impurity **II** using a Hewlett-Packard 1040A detector.

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