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Short Communication

Direct high-performance liquid chromatographic separation of enantiomeric peptidoleukotriene antagonists

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ABSTRACT

Enantiomeric peptidoleukotriene antagonists, SK&F *R*-106203 and SK&F *S*-106203 can be effectively separated on a cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. The utility of this chiral high-performance liquid chromatographic method in assigning absolute stereochemistry to SK&F *S*-106203-Z_2, a non-crystalline amorphous compound which is not amenable to single crystal X-ray analysis, is demonstrated by correlation with the absolute configuration determined crystallographically for a second salt form.

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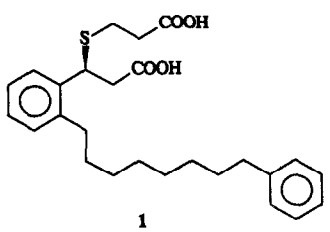
INTRODUCTION

The peptidoleukotrienes (LTs), formed from the metabolism of arachidonic acid by 5'-lipoxygenase, have been the focus of intensive research for their possible roles in the pathophysiology of a variety of disorders [1]. To date, the disease for which the most convincing evidence exists for a primary role of LTs in its etiology is allergic asthma [2]. Most of the pharmacological effects of LTs appear to be receptor-mediated [1] and potent, specific, high-affinity LT receptor antagonists have been identified [3].

SK&F S-106203 {3(*S*)-[2-(carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]propanoic acid} (**1**) is a potent LT receptor antagonist currently in clinical trials. The two enantiomers of this LT receptor antagonist show markedly different pharmacological activities. The *S-enantiomer* (**1**), is more potent than its *R*-enantiomer, as with the close structural analogue, SK&F 104353 {2(*S*)-hydroxy-3(*R*)-[2-(carboxyethyl)-thio]-3-[2-(8-phenyloctyl)phenyl]-propanoic acid} (**2**) which is 100-fold more effective than its enantiomer, SK&F 104373 {2(*R*)-hydroxy-3(*S*)-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl)-phenyl]propanoic acid} in competing for $[^3H]LTD_4$ binding sites on guinea pig lung membranes [4], or for inhibiting LTD_4-induced contractions of guinea pig trachea [5].



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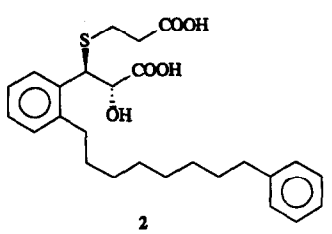

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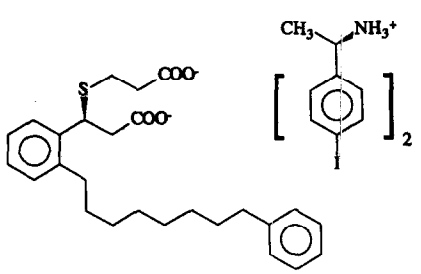

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In order to address the structural assignment of **1** in regulatory submissions [6], and to evaluate the safety and efficacy of the single *S*-enantiomer (**1**), we required a validated stereospecific assay to determine the enantiomeric purity of the drug substance. In this communication, we describe a direct high-performance liquid chromatographic (HPLC) separation of SK&F 106203 racemates, and report on the utility of this chiral separation in assigning absolute stereochemistry to SK&F *S*-106203-Z_2, the disodium salt of **1** which is a non-crystalline amorphous compound not amenable to single crystal X-ray analysis.

EXPERIMENTAL

Solvents were of HPLC grade. All compounds used in this work were prepared in-house. Each compound was characterized by NMR, MS, IR, impurity profile by HPLC and elemental analysis.

Apparatus

The liquid chromatography system consisted of a Beckman Gold HPLC pump connected to an HP 1050 series autosampler and variable-wavelength UV detector. A 25 × 0.46 cm I.D. Chiralcel OD column (J.T. Baker, USA) with cellulose tris(3,5-dimethylphenylcarbamate) (CDMP) coated on silica gel with particle size of 10 μm was used. Data was acquired and processed using a Beckman CIS-CALS laboratory data system.

Chemicals

Racemic SK&F 106203, SK&F *S*-106203-Z_2, and the bis[(*R*)-4-iodo-α-methylbenzenemethanamine] salt of SK&F *S*-106203 **3** were synthesized in-house.

Free SK&F *S*-106203 diacid was liberated from **3** by dissolving 20 mg of the salt in 0.1 *M* hydrochlor-

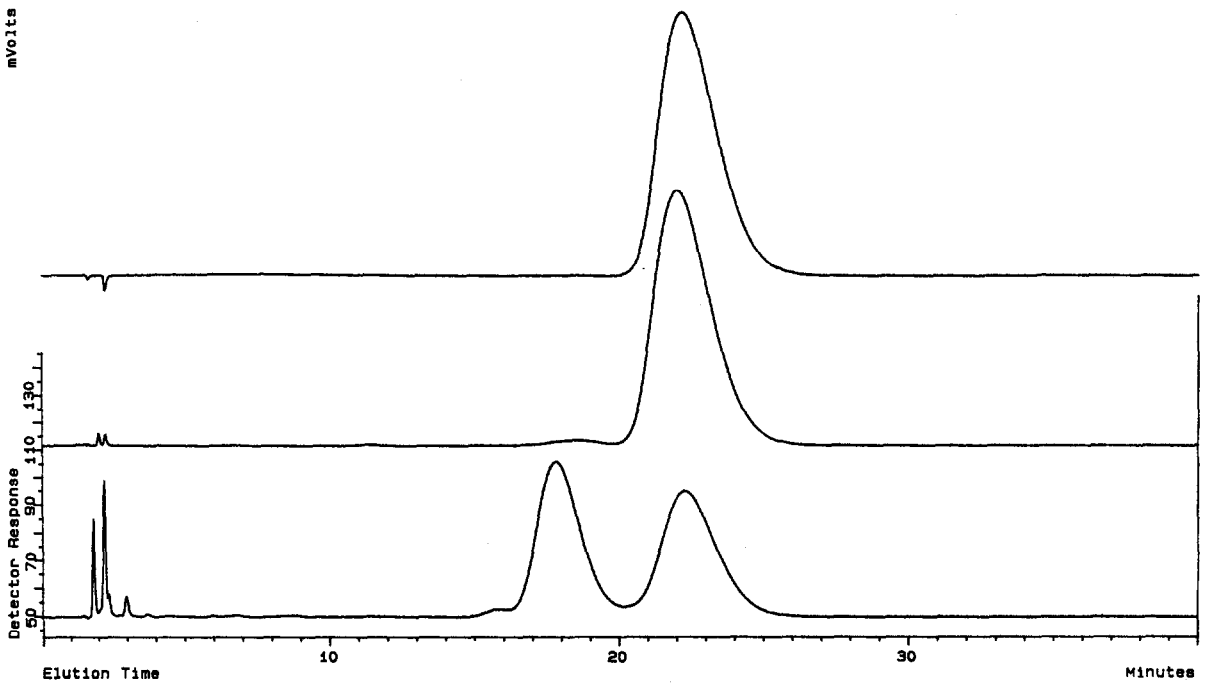

Fig. 1. Chiral HPLC chromatograms of (top trace) SK&F *S*-106203-Z_2, (middle trace) SK&F *S*-106203 free diacid obtained from the bis [(*R*)-4-iodo-α-methylbenzenemethanamine] salt of SK&F *S*-106203 (3) and (bottom trace) racemic (*R*/*S*) SK&F 106203.

ic acid, extracting the free diacid into ethyl acetate, drying under a gently flow of clean nitrogen and reconstitution in mobile phase.

Chromatographic conditions

The mobile phase consisted of a mixture of hexane, 2-propanol and trifluoroacetic acid (96.4:3.5:0.1). The flow-rate was 2 ml/min and UV detection was performed at 215 nm. The sample concentration was *ca.* 0.5 mg/ml in mobile phase with a 100-μl injection loop being used. The chromatography was performed at room temperature.

RESULTS AND DISCUSSION

Direct chiral separation on CDMP stationary phase has been reported for atropine, homoatropine, laudeanosine and tetrahydropalmatine [7], aminoglutethimide [8], β-lactams [9], β-adrenergic blocking alcohols [10–13], abscisic acid [14] as well as N-benzyloxycarbonyl amino acids [15]. The formation of diastereomeric solute–chiral stationary phase complexes through both hydrogen-bonding and dipole–dipole interactions is considered important, and the chiral cavity formed by the carbamate at the 6-position with that at the 2- and/or 3-positions on two neighboring glucose units is thought to play a significant role in chiral recognition [16,17]. Although successful for racemic *para*-substituted 2-arylbutyric acid, such as racemic indobufen [18] and unsubstituted 2-phenylpropionic acid, substituted racemic 2-arylpropionic acids, *e.g.*, ibuprofen, ketoprofen, flurbiprofen and tiaprofenic acid could not be resolved on CDMP stationary phase [17]. However, in the present work, good separation was obtained for racemic (*R*/*S*) SK&F 106203, a 2′,3-disubstituted arylpropionic acid. Separation of other racemic leukotriene antagonists, *e.g.*, 3-(((3-(2-(7-chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-dimethylamino)-3-oxopropyl)thio)methyl)-thio)propionic acid (MK-0571) [19], or the close structural analogue, 5-[3-(2-carboxyethylthio)-1-hydroxypentadeca-3(*E*), 5(*Z*)-dienyl]-phenyl-1-H-tetrazole [20], required either prior derivatization

followed by chiral HPLC or a chiral protein column.

Fig. 1 shows the chiral separation of racemic (*R*/*S*) SK&F 106203. A stereochemical separation factor of 1.5 was obtained. The enantiomeric elution order was determined by performing the chromatography of the *S*-enantiomer, SK&F *S*-106203 under identical conditions. Thus, the peak that eluted with a lower capacity factor was determined as the *R*-enantiomer and the peak with a higher capacity factor, the *S*-enantiomer. The method is precise [relative standard deviation (R.S.D.) 0.28% for six replicate injections] and is linear over the range 0.1 to 4.0% of SK&F *R*-106203 in SK&F *S*-106203 ($y = 9.7910^{e-3}x - 2.9243^{e-4}$, $r = 0.998$). The limit of detection is 0.1% SK&F *R*-106203 in SK&F *S*-106203.

Since SK&F *S*-106203-Z_2 is an amorphous, non-crystalline solid, determination of its absolute stereochemistry via single crystal X-ray crystallography was not possible. However, the absolute stereochemistry of SK&F *S*-106203-Z_2 can be indirectly confirmed by using chiral HPLC to correlate the chirality of SK&F *S*-106203-Z_2 with the more crystalline bis[(*R*)-4-iodo-α-methylbenzenemethanamine] salt of SK&F S-106203 (**3**)] which was amenable to single crystal X-ray analysis. Fig. 1 shows the chiral HPLC chromatrograms of SK&F *S*-106203-Z_2 (top trace), SK&F *S*-106203 free diacid obtained from **3** (middle trace) and racemic (*R*/*S*) SK&F 106203 (bottom trace). As shown in Fig. 1, the chiral chromatography indicated the same absolute stereochemistry for both SK&F *S*-106203-Z_2 and **3**.

CONCLUSIONS

Racemic (*R*/*S*) SK&F 106203, a 2,2-disubstituted arylpropionic acid, can be effectively separated on a chiral tris-(3,5-dimethylphenylcarbamate) stationary phase. The present work also demonstrates the utility of chiral chromatography in assigning absolute stereochemistry to amorphous, non-crystalline forms which may not be amenable to single crystal X-ray analysis.

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