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Note

Chiral separation of the optical isomers of the antimalarial drug halofantrine

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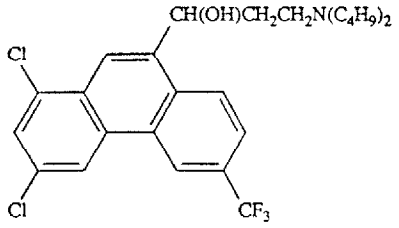
1-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3-N,N-dibutylaminopropan-1-ol (**1**) is a very effective antimalarial agent[1,2]. It is generally known as halofantrine and is currently being marketed by SK&F as the hydrochloride under the trade name of Halfan for the acute treatment of most forms of malaria, even multiple-drug resistant strains.



(**1**)

1 has a chiral centre and the optical isomers have been resolved chemically by fractional crystallisation of the *d*-camphoric acid salts from a hexane–tetrahydrofuran solvent mixture[3].

For halofantrine to be used optimally as an antimalarial agent it is important to evaluate in detail any differences in the pharmacodynamics and the pharmacokinetics of the enantiomers of this drug. To achieve this, it is necessary to have available an analytical method that can distinguish between the two enantiomers with good sensitivity. In this article, we describe a new high-performance liquid chromatography (HPLC) procedure which allows the efficient resolution of these enantiomers using a chiral stationary phase.

EXPERIMENTAL

Chemicals

n-Hexane (Rathburn Chemicals), propan-2-ol, chloroform, ethanol, 2-butanol (BDH) and 99% triethylamine (Aldrich) were filtered through a Millipore Durapore 0.45-μm membrane filter and degassed with helium before use. Racemic halofantrine free base was prepared from halofantrine hydrochloride[4] by the following procedure.

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Halofantrine hydrochloride (13.2 g, 24.6 mmol) was vigorously stirred in 40% (w/v) sodium hydroxide solution (160 ml)/diethyl ether (300 ml) until a solution was formed ($\approx$ 1 h). The alkaline layer was separated from the diethyl ether and was further extracted twice with 150 ml of diethyl ether. The combined ether extracts were dried over magnesium sulphate and filtered. Evaporation of the diethyl ether gave an oil which solidified on addition of dimethyl sulphoxide. Final recrystallisation from nitromethane gave 7.3 g (81%) of **1**, m.p. 83–85°C. Analysis ($C_{26}H_{30}NOCl_2F_3$) calculated C 62.40, H 6.04, N 2.80, Cl 14.17; found C 62.33, H 5.95, N 2.67, Cl 14.41. Mass spectrometry, *m/e* 500 (M^+), 456, 142, 100 (base).

High-performance liquid chromatography

The HPLC method to separate the optical isomers of **1** was developed on a Perkin-Elmer Series 4 liquid chromatograph, equipped with a Perkin-Elmer ISS-100 autoinjector and a Kratos Spectroflow 783 variable-wavelength UV absorbance detector operated at 260 nm. The chiral column (250 × 4.9 mm I.D.) used for the enantiomeric separation was of the Pirkle type and the chromatographic support consisted of L-N-(3,5-dinitrobenzoyl)leucine covalently bound to 3-aminosilica (particle size 5 μm). This column, supplied by Hichrom, was operated at 0°C. The best separation of the enantiomers was achieved using *n*-hexane, chloroform and propan-2-ol (containing 1% triethylamine) in the ratio of 90:5:5 (v/v/v) and flowing at 0.2 ml min^{-1}. UV spectra were recorded on a Hewlett-Packard 1040A diode array detection system.

Specific rotation $[\alpha]_D^T$

$[\alpha]_D^T$ of collected fractions was determined in *tert.*-butyl methylether using a Perkin-Elmer 241 polarimeter set at the sodium D-line (589 nm). Optical rotation was determined in a cell of 100 mm pathlength, thermostatted at 25°C.

RESULTS AND DISCUSSION

The chromatogram in Fig. 1 shows the chiral resolution of the optical isomers of halofantrine using the conditions detailed in the Experimental Section. As expected, the UV absorbance of the separate enantiomers is identical to that of halofantrine itself. The (+)-enantiomer elutes first with a retention time of 24.3 min followed by the (+)-enantiomer at 26.1 min. The separation factor α is equal to 1.28.

A number of different chiral resolution chromatographic methods, utilising a varicty of column supports (Chiralcel OC, OJ and OF, Resolvosil-BSA-7 and Cyclobond I) were used. These columns did not give any degree of separation. A number of different solvent system combinations were also tried on the L-leucine column. For example, replacing propan-2-ol with either ethanol or 2-butanol gave no enantiomeric separation. Moreover, the tertiary amino group of **1** appears to interact strongly with the silanol groups in the column packing so that excluding triethyl amine as a mobile phase additive[5] gave broader peaks, longer retention times and poor enantiomeric resolution.

Repeat injection and collection of two fractions, one from the beginning of elution of the first peak to the same apex and another from the second peak to the end of elution, gave (on evaporation of the solvent) sufficient quantities of the two

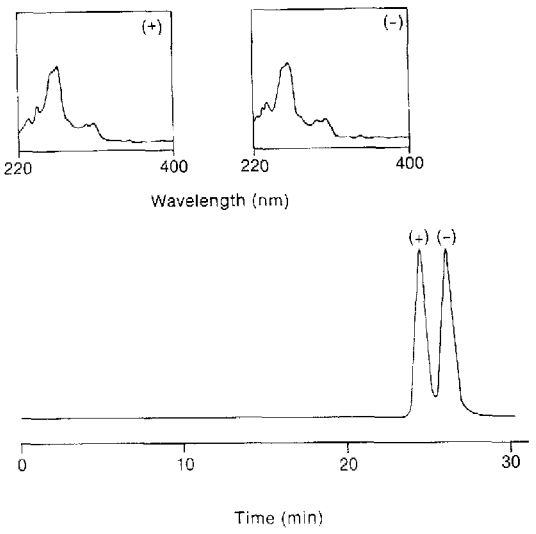

Fig. 1. Enantiomeric separation of the optical isomers of halofantrine **1**. Inserts show the UV spectra of the (+)- and (−)-enantiomers.

enantiomers for polarimetric analysis. This identified the first and second fractions as containing predominantly the (+)- and (−)-enantiomers, respectively. The optical rotation $[\alpha]_D^{25}$ of these fractions were measured as +38° and −24° respectively. These rotations are of a similar order of magnitude to those (about 41°) reported in the literature for the enantiomers of **1** resolved by complex formation with *d*-camphoric acid[3].

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