Journal of Chromatography, 615 (1993) 347–351
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6803

Short Communication

Direct determination of the enantiomers of halofantrine and its pharmacologically active metabolite N-desbutylhalofantrine by high-performance liquid chromatography

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(First received November 13th, 1992; revised manuscript received March 1st, 1993)

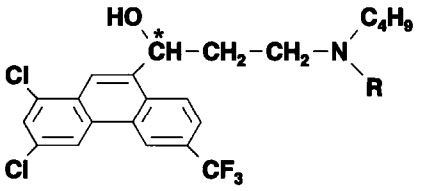
ABSTRACT

The enantiomers of halofantrine and its main metabolite, N-desbutylhalofantrine, were separated on two different polysaccharide-based chiral stationary phases. The stereoselective biotransformation of halofantrine was determined using rat liver homogenates. (−)-Halofantrine is metabolized preferentially.

INTRODUCTION

Halofantrine, 1-(1,3-dichloro-6-trifluoromethylphenanthryl)- 3-N,N-dibutylaminopropan-1-ol (Fig. 1), a chiral phenanthrene methanol derivative, is an orally administered blood schizontocide, active against both chloroquine-sensitive and chloroquine-resistant plasmodia. The drug is marketed as the racemate. Its main metabolite, N-desbutylhalofantrine (Fig. 1), is also pharmacologically active [1,2]. The pharmacokinetics of racemic halofantrine and its metabolite N-desbutylhalofantrine has been investigated [3,4].

The separation of the halofantrine enantiomers on a Pirkle-type chiral stationary phase with L-N-(3,5-dinitrobenzoyl)leucine covalently bound to 3-aminopropylsilica has been reported [5]. Recently, the stereoselective metabolism of halofantrine was also investigated. Before the determination of the enantiomeric ratio of halofantrine and N-desbutylhalofantrine, both



R= C_4H_9 : Halofantrine

R= H : N-Desbutylhalofantrine

Fig. 1. Structures of halofantrine and N-desbutylhalofantrine.

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0378-4347/93/$06.00

compounds had to be separated by achiral reversed-phase liquid chromatography. Subsequently, the fractions of halofantrine and N-desbutylhalofantrine were collected and resolved into their enantiomers on an ovomucoid chiral stationary phase [6].

In this communication we describe the direct chiral resolutions of both halofantrine and N-desbutylhalofantrine without previous fractionation. Using these chromatographic conditions the stereoselective biotransformation of halofantrine was investigated *in vitro*.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Merck Hitachi L-6000 pump, a Merck Hitachi L-4000 UV detector operated at a wavelength of 260 nm, a Merck Hitachi 655-61 processor, Chiralcel OD and Chiralpak AD analytical columns containing cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel and amylose tris-3,5-dimethylphenylcarbamate coated on silica gel, each (250 mm × 4.6 mm I.D., 10 μm particle size) equipped with the corresponding guard columns (50 mm × 4.6 mm I.D., 10 μm particle size), all columns purchased from Baker (Gross-Gerau, Germany).

Chemicals

Racemic halofantrine and the metabolite N-desbutylhalofantrine were gifts from SK&F (Welwyn Garden City, UK). Camphoric acid was purchased from Fluka (Buchs, Switzerland), the HPLC solvents and NADPH (dihydronicotinamide adenine dinucleotide phosphate) were purchased from Merck (Darmstadt, Germany). For comparison the enantiomers were prepared using camphoric acid as the resolving agent [7] using methyl ethyl ketone as a solvent for recrystallization.

Chromatographic conditions

The mobile phase of the Chiralcel OD column consisted of *n*-hexane–2-propanol–diethylamine (90:10:0.1, v/v); the mobile phase of the Chiralpak AD column was *n*-hexane–ethanol–2-butanol–diethylamine (93:4.5:2.5:0.1, v/v), operated at a flow-rate of 0.3 ml/min.

In vitro biotransformation studies

For *in vitro* studies the supernatant of liver homogenates of phenobarbitone-induced rats prepared as described previously [8] was used. A 250-μl volume of the supernatant was added to a suspension of 10 μg of halofantrine racemate in 1.0 ml of Tris buffer (0.1 *M*, pH 7.8) and 100 μl of 0.06 *M* magnesium chloride solution. The samples were incubated for 4 h at 37°C. A 100-μl aliquot of 10 m*M* NADPH was added at 1-h intervals. Following the incubation the samples were adjusted to pH 9 by addition of 0.1 *M* sodium hydroxide. A 3.5-ml aliquot of *n*-hexane–diethyl ether (70:30, v/v) was added and mixed on a mechanical shaker for 15 min. The suspensions were centrifuged at 2500 *g* for 15 min, and the organic phase transferred into siliconized tubes. This extraction step was repeated in order to ensure a complete extraction. The combined fractions were dried at 40°C under a stream of nitrogen. The residue was dissolved in 1 ml of the mobile phase. Aliquots of 20 μl were injected into the HPLC system.

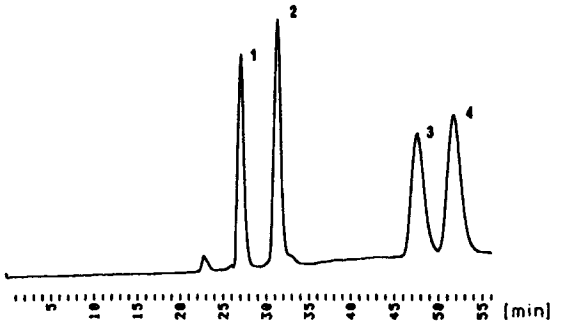

Fig. 2. Separation of racemic halofantrine and N-desbutylhalofantrine on Chiralcel OD. Mobile phase: *n*-hexane–2-propanol–diethylamine (90:10:0.1, v/v); flow-rate: 0.3 ml/min. Sample: mixture of racemic halofantrine and N-desbutylhalofantrine, each 5 μg/ml. Injection volume: 20 μl. Peaks: 1 = (+)-halofantrine; 2 = (−)-halofantrine; 3 and 4 = enantiomers of N-desbutylhalofantrine.

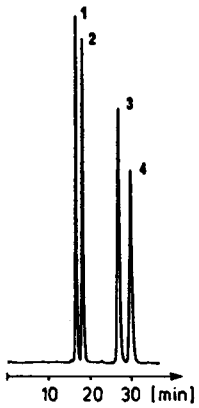

Fig. 3. Separation of racemic halofantrine and N-desbutylhalofantrine on Chiralpak AD. Mobile phase: *n*-hexane–ethanol–2-butanol–diethylamine (93:4.5:2.5:0.1, v/v); flow-rate: 0.3 ml/min. Sample: mixture of racemic halofantrine and N-desbutylhalofantrine, each 5 μg/ml. Injection volume: 20 μl. Peaks: 1 = (+)-halofantrine; 2 = (−)-halofantrine; 3 and 4 = enantiomers of N-desbutylhalofantrine.

RESULTS AND DISCUSSION

Analytical separations

The complete chiral resolution of halofantrine and its metabolite N-desbutylhalofantrine without prior achiral separation was accomplished on Chiralcel OD and Chiralpak AD columns (Figs. 2 and 3). The standard mobile phase for the columns, *n*-hexane–2-propanol (90:10, v/v), enabled a good separation of halofantrine enantiomers, whereas N-desbutylhalofantrine was not resolved. The optimal mobile phase system for resolution of halofantrine and its metabolite was found to be *n*-hexane–2-propanol–diethylamine (90:10:0.1, v/v) on Chiralcel OD and *n*-hexane–ethanol–2-butanol–diethylamine (93:4.5:2.5:0.1, v/v) on Chiralpak AD, respectively.

In vitro biotransformation studies

The biotransformation of halofantrine *in vitro* was investigated using phenobarbitone-induced rat liver microsomal and supernatant fractions. Significant biotransformation was observed only with the supernatant. After incubation the concentration of (+)-halofantrine was found to be higher than that of (−)-halofantrine. This result correlates with the result of Gimenez *et al.* [6], who found (+)-halofantrine in a higher concentration than (−)-halofantrine in a plasma concentration curve. N-Desbutylhalofantrine is also formed predominantly from the (−)-enantiomer of halofantrine. In two blanks, the first containing supernatant and all additives except the substrate and the second Tris buffer and all additives

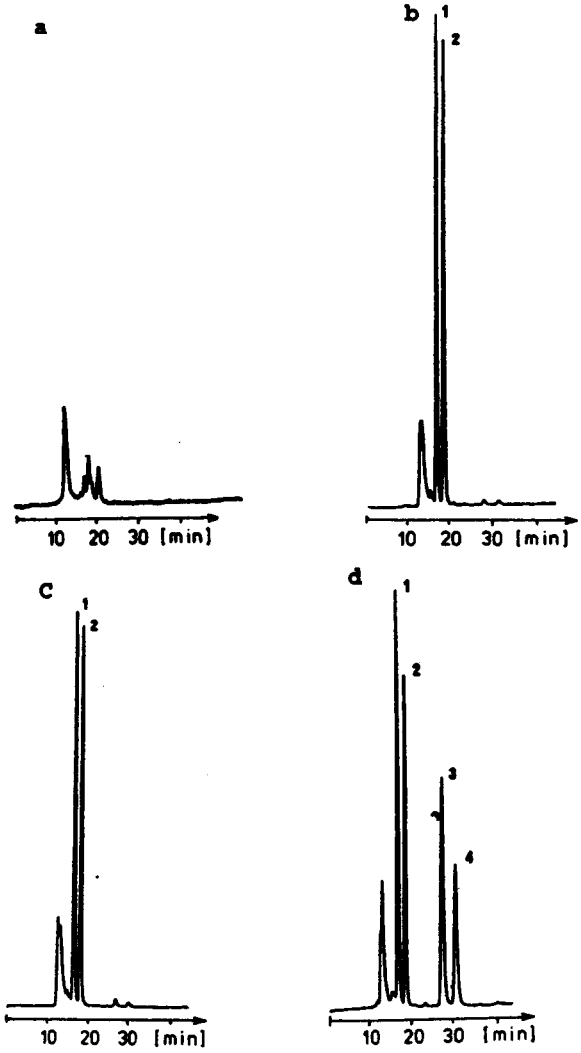

Fig. 4. Chromatograms of the biotransformation of racemic halofantrine from induced rat liver supernatant on Chiralpak AD. (a) Supernatant and all additives except substrate. (b) Tris buffer, substrate and all additives except supernatant; peaks: 1 = (+)-halofantrine; 2 = (−)-halofantrine. (c) Incubation without NADPH; peaks: 1 = (+)-halofantrine; 2 = (−)-halofantrine. (d) Incubation with NADPH; peaks: 1 = (+)-halofantrine; 2 = (−)-halofantrine; 3 and 4 = enantiomers of N-desbutylhalofantrine. For chromatographic conditions, see Experimental section.

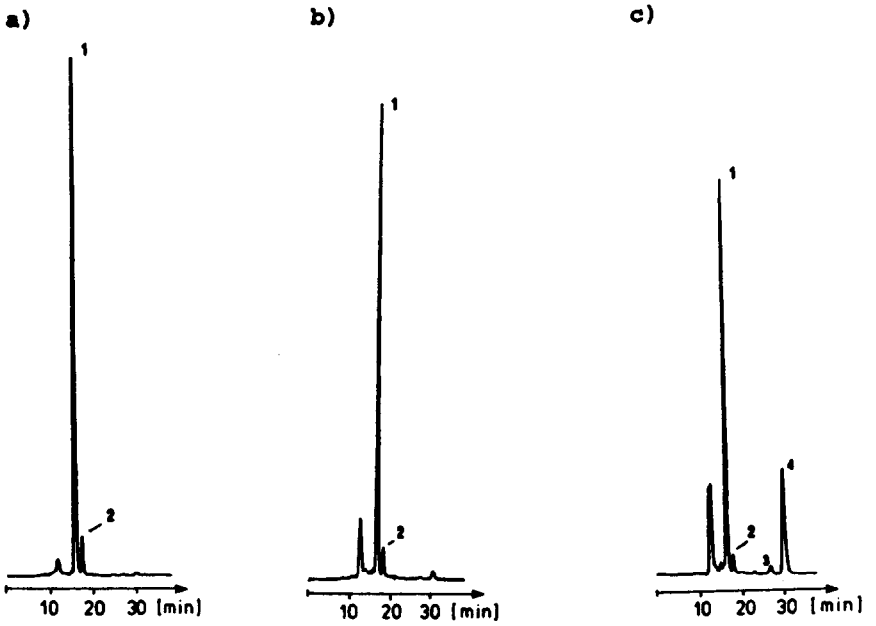

Fig. 5. Chromatograms of the biotransformation of the (+)-halofantrine enantiomer on Chiralpak AD. (a) Tris buffer and all additives except supernatant; peaks: 1 = (+)-halofantrine (enantiomeric excess, ee = 98%); 2 = (−)-halofantrine as an impurity. (b) Incubation without NADPH; peaks: 1 = (+)-halofantrine (ee = 98%); 2 = (−)-halofantrine as an impurity. (c) Incubation with NADPH; peaks: 1 = (+)-halofantrine (ee = 98%); 2 = (−)-halofantrine as an impurity; 3 = N-desbutylhalofantrine enantiomer, metabolite of (−)-halofantrine; 4 = N-desbutylhalofantrine enantiomer, metabolite of (+)-halofantrine. For chromatographic conditions see Experimental section.

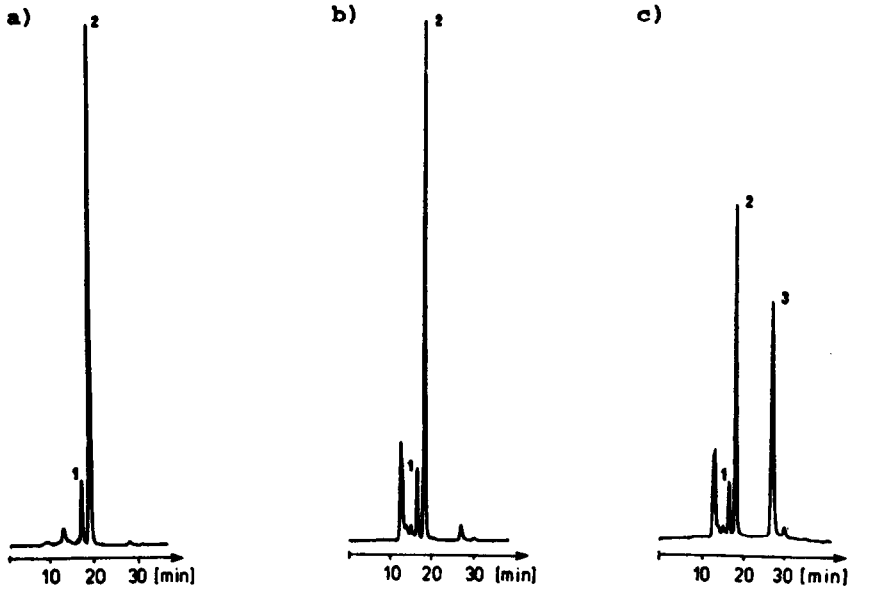

Fig. 6. Chromatograms of the biotransformation of the (−)-halofantrine enantiomer on Chiralpak AD. (a) Tris buffer and all additives except supernatant; peaks: 1 = (+)-halofantrine as an impurity; 2 = (−)-halofantrine (ee = 94%). (b) Incubation without NADPH; peaks: 1 = (+)-halofantrine as an impurity; 2 = (−)-halofantrine (ee = 94%). (c) Incubation with NADPH; peaks: 1 = (+)-halofantrine as an impurity; 2 = (−)-halofantrine (ee = 94%); 3 = N-desbutylhalofantrine enantiomer, metabolite of (−)-halofantrine. For chromatographic conditions see Experimental section.

except the supernatant, no peak of N-desbutylhalofantrine was observed, and in the latter case the concentrations of (+)- and (−)-halofantrine were equal (Fig. 4).

In order to determine enantiomeric elution orders, both enantiomers of halofantrine were incubated separately under the same conditions. N-Desbutylhalofantrine formed from (−)-halofantrine was eluted first (Fig. 5) and N-desbutylhalofantrine formed from (+)-halofantrine as a second peak (Fig. 6). Therefore the elution order of the enantiomers of halofantrine/N-desbutylhalofantrine on Chiralpak AD is reversed. In order to prove that this reversal is not because of retention time change, the two samples were mixed with racemic N-desbutylhalofantrine and injected. As a result the peaks obtained were larger.

Further investigations on the stereoselective biotransformation of halofantrine are in progress.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. Racemic halofantrine and N-desbutylhalofantrine were gifts from Smith Kline and French Research Limited.

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