Investigation of the metabolism of 7-(4-chlorbenzyl)-7,8,13,13a-tetrahydroberberine chloride in the rat

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SUMMARY

The metabolism of 7-(4-chlorbenzyl)-7,8,13,13a-tetrahydroberberine chloride (CTHB), a compound with promising pharmacological effects against arrhythmia, was investigated in rat bile. A metabolite and unchanged CTHB were found in the bile. Characterization and structural elucidation of the metabolite was achieved by LC/MS and LC/NMR. The following metabolic pathway is proposed: CTHB is metabolized by demethylation at position 10 to produce a new entity.

![Structure and proposed metabolic pathway of CTHB.](image)

INTRODUCTION

7-(4-chlorbenzyl)-7,8,13,13a-tetrahydroberberine

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MATERIALS AND METHODS

Chemicals

CTHB was synthesized and purified by the Research Division of Medicinal Chemistry at the China Pharmaceutical University and was judged to be pure by HPLC. All the chemical shifts of $^{13}$C and protons were assigned by H-H COSY, C-H COSY, HMQC and HMBC. All solvents and reagents were analytical grade.

Animal experiments

Male Sprague-Dawley rats (approximately 200 g) were anesthetized with pentobarbital sodium. Bile was collected in ice-cooled tubes after intravenous administration of CTHB (4 mg/kg). Part of the endogenous bile components were precipitated by adding three volumes of methanol to one volume of bile, the supernatant samples were concentrated and analyzed.

LC/MS

LC/MS was performed on a Finnigan LC/MS system. Finnigan MAT SSQ710 mass spectrometer was coupled to a ESI interface. The LC system consisted of a 4100MS pump and 4.6 x 250 mm ODS-C$_{18}$ column with the mobile phase of MeOH:H$_2$O:HAc: (C$_2$H$_5$N (60:40:0.03:0.04, v/v).

LC/NMR

The stopped-flow $^1$H-NMR detection of the LC eluents was carried out using a Bruker DRX 400 MHz NMR spectrometer equipped with an H/C/D 4 mm NMR-LC probe (z-gradient) The mobile phase was MeOH:D$_2$O:HAc:(C$_2$H$_5$N (60:40:0.03:0.04, v/v) at a flow rate of 1 ml/min. Solvent suppression was achieved by using the presaturation and Watergate double suppression procedure (1).

RESULTS AND DISCUSSION

Figure 2 shows the total ion chromatograph (a) and mass spectra of peak A (b) and peak B (c). Obviously, the ion at $m/z$ 464.2 must be the ion of CTHB ([C$_{27}$H$_{27}$ClNO$_4$]$^+$, peak B), and the ion at $m/z$ 450.2 is produced by the metabolite (peak A). The data indicate that one of the two methoxyl groups (9-OCH$_3$ and 10-OCH$_3$) of CTHB is demethylated.

The $^1$H NMR spectra of CTHB and the metabolite are given in Figures 3 and 4, respectively. Figure 5 shows the difference of $^1$H spectra of CTHB and the metabolite. The assignments of $^1$H spectra lines of CTHB and the metabolite are as follows:

CTHB

H$_1\delta$ 7.204, H$_4\delta$ 7.086; H$_{11}\delta$ 7.403, H$_{12}\delta$ 7.441 (J = 9.39 Hz); H$_c\delta$ 7.365, H$_d\delta$ 7.660 (J = 8.80 Hz).