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Influence of different types of cyclodextrins on the racemization of scopolamine-N-butylbromide

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Abstract

The influence of cyclodextrins (CDs) on the racemization and hydrolysis of scopolamine-N-butylbromide was determined kinetically. α-CD and γ-CD enhance the racemization rate of the substance. β-CD shows no effect and DM-β-CD (dimethyl-β-CD) causes inhibition of racemization. The hydrolysis of the substance was retarded by β-, γ- and DM-β-CD. In the presence of α-CD no change in hydrolysis rate was observed.

Keywords: Racemization; Mobile-phase composition; Enantiomer separation; Cyclodextrins; Scopolamine-N-butylbromide; Tropic acid

1. Introduction

The ability of cyclodextrins to influence isomerization of stereoisomers is known from the literature. Aso et al. [1] described the inhibition of the racemization of ethiazide and the epimerization of etoposide by DM-β-CD. γ-CD retarded the racemization of etoposide and accelerated the racemization of carbenicillin. Blaschke et al. [2] reported the influence of different types of CD at pH 6 and 60°C on the hydrolysis and racemization of the alkaloids (*S*)-hyoscyamine and (*S*)-scopolamine. All CDs used except α-CD retarded hydrolysis and racemization of the substances. The stability to racemization and hydrolysis increased in the following order: γ-CD, β-CD, hydroxypropyl-β-CD, DM-β-CD. Chiral media other than CDs can affect isomerization reactions. The influence of human serum albumin (HSA) on the epimerization or racemization of carbenicillin, ethiazide and etoposide was investigated. HSA retarded the racemization of ethiazide and the epimerization of etoposide and accelerated the epimerization of carbenicillin [3]. HSA enhanced the isomerization rate of ceftibuten [4]. Also liposomes can affect the racemization rate of chiral compounds. The racemization rate of chlortalidon decreased under the influence of liposomes [5]. Racemization of scopolamine-N-butylbromide takes place in alkaline solution. In this study, the effect of different types of cyclodextrins on the racemization and the hydrolysis of scopolamine-N-butylbromide was examined kinetically.

2. Experimental

2.1. Instrumentation

The HPLC system was a LiChrograph Merck-Hitachi consisting of an L-6000 pump, an L-4200

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UV–Vis detector, a T-6300 column thermostat, a 7125 Rheodyne injector with 20-μl loop and a D-2500 Chromato Integrator (Merck, Darmstadt, Germany). The UV spectra were measured with a diode-array detector of LC 1090 II (Hewlett-Packard, Waldbronn, Germany).

Separation was achieved using a Chiralcel OD-R column (25 cm×0.46 cm I.D.; 10 μm; Daicel Chemical Industries, Tokyo, Japan).

Solid-phase extraction was carried out with a Baker SPE-12G system.

2.2. Chemicals

Buscopan (scopolamine-N-butylbromide) was obtained from Boehringer (Ingelheim, Germany). $NaClO_4$, methanol, H_2SO_4 were purchased from Laborchemie Apolda (Germany), α-, γ-, DM-β-CD from Gyógyszertechnológiai Intézet Hallgatók Laboratóriuma Szeged (Hungaria), β-CD from Serva (Heidelberg, Germany) and acetonitrile (LiChrosolv) from Merck. The water used was doubly distilled.

2.3. Analytical chromatography

The concentrations of scopolamine-N-butylbromide enantiomers were determined by enantioselective HPLC (Fig. 1). A Chiralcel OD-R column was used as chiral stationary phase. The resolution of the substance with this column has been described in the literature [6].

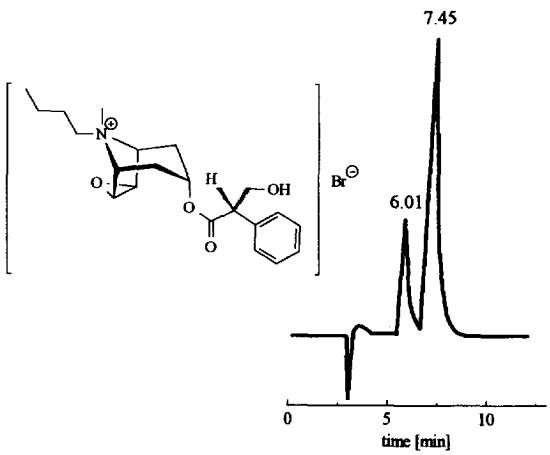

Fig. 1. Chromatogram of scopolamine-N-butylbromide. Stationary phase, Chiralcel OD-R; mobile phase, 1 *M* $NaClO_4$ (pH 2.0)–acetonitrile (6:4); flow-rate, 1 ml/min.

The mobile phase was a mixture of 1 *M* $NaClO_4$ buffer (pH 2) and acetonitrile (6:4). The detection wavelength was 220 nm, the flow-rate was 1 ml/min, and the separation factor was 1.4.

Because no racemate of scopolamine-N-butylbromide was available, the chiral separation was tested by injecting a solution of the (*S*)-enantiomer that contained amounts of (*R*)-enantiomers formed by racemization under test conditions. To confirm the assumption that the second peak in the chromatogram was the (*R*)-enantiomer, the UV spectra of both enantiomers were compared. The two peaks obtained in the chromatogram had the same UV spectrum. In accordance with the literature the (*R*)-enantiomer eluted first [6]. The formation of the hydrolysis product tropic acid was verified by injection of a reference solution of tropic acid.

2.4. Solid-phase extraction

It was necessary to find an extraction method for the substance, because the racemization was investigated in alkaline solutions which could not be injected onto the HPLC column. A solid-phase extraction method with ion-exchange columns (Baker SPE* column: carboxylic acid, 1 ml) was used to isolate scopolamine-N-butylbromide from the alkaline buffer solution. The column was activated with 3 ml of methanol and 3 ml of water. Then 0.5-ml samples were applied. CDs were removed from the column with 3 ml of water. The analyte was eluted with 1 ml of the same buffer solution as contained in the mobile phase for HPLC separation. The eluate was subjected to HPLC.

3. Results and discussion

3.1. Racemization

(*S*)-Scopolamine-N-butylbromide was found to convert to the (*R*)-enantiomer and hydrolyzed in

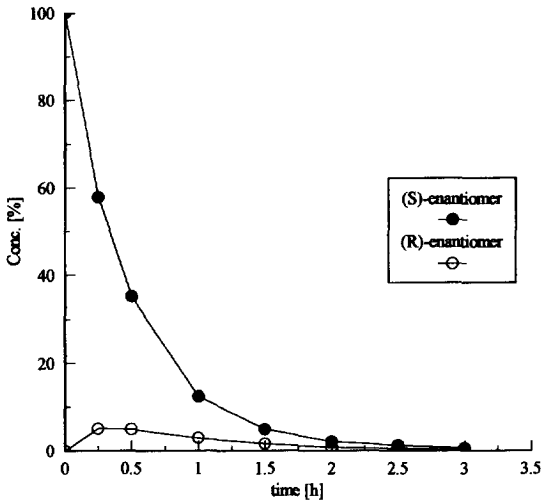

Fig. 2. Time courses of (*S*)- and (*R*)-enantiomer concentrations in the absence of CD.

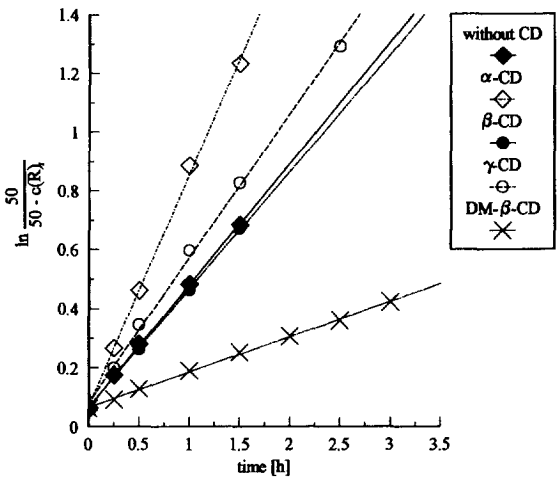

Fig. 3. Racemization of scopolamine-N-butylbromide in the presence of CDs.

alkaline solutions (Fig. 2). The behaviour of the substance was examined in a Delory/King buffer solution (containing Na_2CO_3 and $NaHCO_3$ [7]) of pH 9.7 at a temperature of 50°C. Of the (*S*)-enantiomer 28% was converted to the (*R*)-enantiomer within 3 h. Due to the fast degradation of the substance, complete racemization could not be observed.

The effects of the following CDs were examined: α-, β-, γ- and DM-β-CD. The molar ratio between CD and scopolamine-N-butylbromide was 10:1.

The concentration ratio of the enantiomers was estimated. CDs affect the racemization rate of scopolamine-N-butylbromide: α-CD and γ-CD increase the racemization rate; DM-β-CD causes a strong inhibition; β-CD has no effect on racemization. Racemization is a reversible first-order reaction. If the equilibrium concentrations of both enantiomers are 50%, integration of the appropriate rate equation yields:

$$\ln \frac{50}{50 - c(R)_t} = 2kt \qquad (1)$$

where $c(R)_t$ represents the concentration of the *R*-isomer.

The plot of $\ln [50/(50 - c(R)_t)]$ against time gives a straight line (Fig. 3).

An achiral enol structure is assumed to be an intermediate in racemization. Formation of enols is catalyzed by hydroxide ions. In the case of hyoscyamine racemization an achiral enol intermediate is supposed [8]. The same mechanism of racemization may be assumed for scopolamine-N-butylbromide, because both substances are tropic acid esters. The formation of inclusion complexes of CD and scopolamine-N-butylbromide can explain the retardation of racemization, i.e. the included molecules are protected from the hydroxide ions in the reaction solution. However, the cavity of α-CD seems to be too small for a sufficient protection. Only a part of the molecule may be included in the CD.

The secondary hydroxyl groups of CD can catalyze reactions such as ester hydrolysis, which can take place because of the close distance of the ester group to the secondary hydroxyl groups of the CD [9].

The included molecules have presumably different positions in the α-, β-, and γ-CD, respectively, due to the different sizes of the cavities. The scopolamine-N-butylbromide molecule could be included in the CD cavity in such a way that the secondary hydroxyl groups of the CD are close to the chiral C-atom thus accelerating racemization by catalyzing the formation of the enolstructure. CDs

facilitate the formation of enols [10]. The fixation of the molecule in the large cavity of γ-CD is possibly not strong enough to support the catalytic effect of secondary hydroxyl groups. In the case of DM-β-CD one of the secondary hydroxyl groups is methylated. This fact can explain the strong inhibition of racemization by DM-β-CD.

Inhibition by all CDs used is described for the racemization of scopolamine at pH 6 [2].

3.2. Hydrolysis

Since the hydrolysis product tropic acid was found in the solution, scopolamine-N-butylbromide undergoes hydrolysis under the above conditions.

Hydrolysis of scopolamine-N-butylbromide follows a first order reaction. The logarithmic plot of concentration against time shows a straight line (Fig. 4). Integration of Eq. 2 yields Eq. 4. Hydrolysis of the racemate was determined. Concentration at time t is the sum of the concentrations of the (*S*)- and (*R*)-enantiomers with regard to an initial concentration of 100% (Eq. 3).

$$-\frac{dc_t}{dt} = kt \tag{2}$$

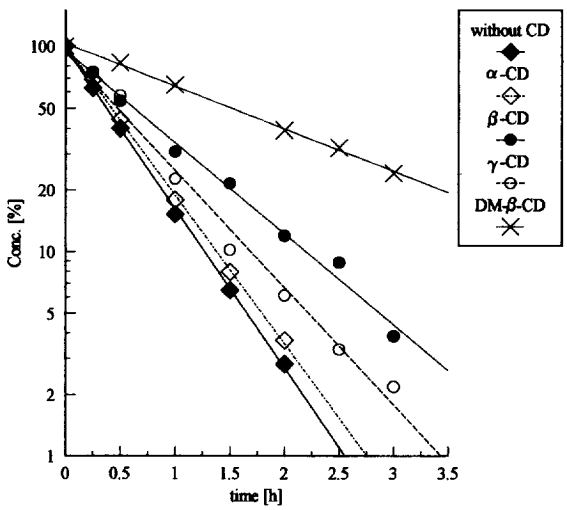

Fig. 4. Hydrolysis of scopolamine-N-butylbromide in the presence of CDs.

$$c_t = c(S)_t + c(R)_t \tag{3}$$

$$-k = \frac{\ln [c(S)_t + c(R)_t] - \ln 100}{t} \tag{4}$$

The hydrolysis rate changed in the presence of CDs (Fig. 4). All CDs used decelerated the reaction rate. β-CD and γ-CD inhibited hydrolysis. DM-β-CD enhanced the 50%-hydrolysis time 4-fold. α-CD did not give a substantial change in the hydrolysis rate.

The inhibition of hydrolysis by CDs can be explained by the complete inclusion of scopolamine-N-butylbromide as in the case of racemization. The CD protects the ester group from the attack of hydroxide ions in the solution. The effect of α- and β-CD on the hydrolysis of atropine in alkaline solution (pH 10) was examined by Chin et al. [11]. They observed an acceleration of hydrolysis in the presence of α-CD and a deceleration in the presence of β-CD.

Complete inclusion of the ester group in the cavity of the CD with simultaneous acceleration of the racemization by the secondary hydroxyl groups is only possible if not the phenyl ring of the tropic acid, but the tropine part of the molecule is included in the CD cavity. The chiral C-atom must be located outside the cavity close to the secondary hydroxyl groups, as postulated in a former study on the hydrolysis of 2-methoxy-2-phenylacetic acid-nitrophenyl esters [12]. The true position of scopolamine-N- butylbromide could not be established in this study.

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