

In Vitro Nitrosation of Ephedrine in the Presence of Cyclodextrins[★]

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Abstract. β -, and γ -cyclodextrin and heptakis-2,6-di-*O*-methyl- β -cyclodextrin enhance the nitrosation rate of *l*-ephedrine if the nitrosation assay procedure (NAP test) is applied. During this reaction with β -cyclodextrin a solid inclusion compound of β -cyclodextrin and *N*-nitrosoephedrine precipitates. Solubilities and stabilities of inclusion compounds of the cyclodextrins with ephedrine and nitrosoephedrine, respectively, explain especially the catalytic effects of some cyclodextrins on ephedrine.

Key words: Nitrosation reactions: α -, β -, γ -cyclodextrin, dimethyl- β -cyclodextrin, ephedrine, nitrosoephedrine.

1. Introduction

β - and γ -cyclodextrin (CD) and heptakis-2,6-di-*O*-methyl- β -cyclodextrin (DIMEB) catalyze significantly the *in vitro* nitrosation of the slowly nitrosatable ephedrine, if the nitrosation assay procedure (NAP test) is applied [1]. During the procedure with β -CD a precipitate is formed under the conditions used after about 120 minutes. The formation of a precipitate was also observed in the nitrosation of fencamfamine in the presence of γ -CD [1]. It was identified as the inclusion compound of nitrosofencamfamine with γ -CD.

It is the purpose of this paper to examine the composition of the precipitate and to discuss the mechanism of the increased nitrosation reaction.

2. Materials and Methods

α -CD: Aldrich Europe, Beerse; β -CD: Chinoïn Co., Budapest; γ -CD: Nihon Shokuhin Kako Co., Tokyo. DIMEB: Prepared according to Szejtli *et al.* [2]; Fp. 311°C. The NMR spectrum corresponds to the specifications given by Casu *et al.* [3]. *l*-Ephedrine sulfate: Serva Co., Heidelberg. *N*-nitrosoephedrine has been prepared according to Kinawi and Schuster [4]. Nitrosation procedures were performed as described in [1].

¹H-NMR spectra were taken on a 250 MHz spectrometer, WM 250 (Bruker Co., Karlsruhe). The internal standard was 3-trimethylsilylpropionic acid-*d*₄-sodium.

2.1. INCLUSION COMPOUND OF β -CD AND NITROSOEPHEDRINE

A solution of 100 mg β -CD in 4.0 ml of a solution which contains 4.7135 g *l*-ephedrine sulfate and 1.0 ml HCl (37%) in 1000 ml water (37°C) was mixed vigorously with 400 μ l of a solution of 3.036 g sodium nitrite in 100 ml water (37°C) in a small screw-topped 4.5-ml plastic tube.

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After 120 min the precipitate was centrifuged at 3000 rpm for 3 min. After decantation of the supernatant fluid, the precipitate was dried at 40°C on filter paper. The yield was 10 mg of a white powder.

3. Results

Some possibilities exist concerning the composition of the precipitate. It could be the inclusion compound of β -CD and ephedrine, pure nitrosoephedrine (NEPH) or the inclusion compound of β -CD with NEPH. We were unsuccessful in isolating the solid inclusion compound between β -CD and ephedrine by this method. No formation of solid NEPH could be observed in the absence of CD. We assumed the formation of the inclusion compound of β -CD and NEPH.

To prove this, the $^1\text{H-NMR}$ spectra of the following samples were examined:

- (A) β -CD
- (B) An equimolar mixture of ephedrine and β -CD
- (C) The precipitate from the reaction mixture
- (D) An equimolar mixture of NEPH and β -CD

The spectrum of the precipitate (C) was not different from that of the mixture of NEPH and β -CD (D). This demonstrates that the precipitate is composed of NEPH and β -CD.

From the integration curve of spectrum C the molar ratio of 1 : 1 has been determined. Obviously the precipitate is an inclusion compound.

The nitrosation of ephedrine is catalyzed significantly by β -CD, γ -CD and DIMEB, but not by α -CD. The initial reaction rates increase in the relative order

$$k_{\text{without}} < k_{\gamma} < k_{\beta} < k_{\text{DIMEB}} = 1 : 2.3 : 3.4 : 7.4 \quad (1)$$

β -, γ -CD and DIMEB can form soluble inclusion compounds with ephedrine (Table I). The weakest interaction exists between γ -CD and ephedrine. Therefore, a relation is assumed

TABLE I. Chemical shifts ($\Delta\delta$) of the protons of β - and γ -CD and DIMEB in the presence of ephedrine (in D_2O)

CD-proton	β -CD	γ -CD	DIMEB
H-1	0.0011	0.0043	0.0055
H-2	0.0265	0.0083	0.0056
H-3	-0.0587	-0.0034	-0.0408
H-4	0.0051	0.0042	0.0081
H-5	-0.0912	-0.0098	-0.0011
H-6	-0.0110	-0.0032	-0.0340

between the inclusion formation in solution and the catalytic effects regarding the nitrosation reaction.

The reason for the increased activity is reflected in the different solubilities and stabilities of the inclusion compounds formed. Dissolved CD can react with amine and nitrosamine, respectively. Because of the reduced polarity of the nitrosamine compared to the amine, the tendency to interact with β -CD should be greater for the nitrosamine than for the ephedrine.

Additionally, the solubility product of the inclusion compound with NEPH seems to be smaller than that with ephedrine. The precipitation of the β -CD-NEPH inclusion compound results in a change of the reaction equilibrium. This favors an increased reaction rate of the nitrosation process.

The catalytic effects of CDs do not only depend on the formation of a solid inclusion compound, as can be seen by the reaction with γ -CD and DIMEB: The inclusion compounds of these two CDs with NEPH are more soluble than the corresponding adduct with β -CD. The catalytic effects of CDs should also depend on the stability constants of the dissolved inclusion compounds. DIMEB with its more hydrophobic cavity should form a more stable complex with guest molecules than β -CD and γ -CD. It also has the strongest catalytic effect concerning ephedrine nitrosation.

Because of the limited chemical stability of NEPH the formation constant cannot be determined by the solubility method. But NMR spectroscopy of the CD protons can provide an estimate. Thakkar and Demarco [5] reported a correlation between the values of the formation constant, determined by the solubility method [6], and the shift of H-5 induced by barbiturate substrates.

Figure 1 shows the dependence of the shifts of H-3 and H-5 on the substrate/ β -CD ratio in the dissolved complexes of ephedrine and NEPH. The ratios were 0.1 : 1, 0.25 : 1, 0.5 : 1

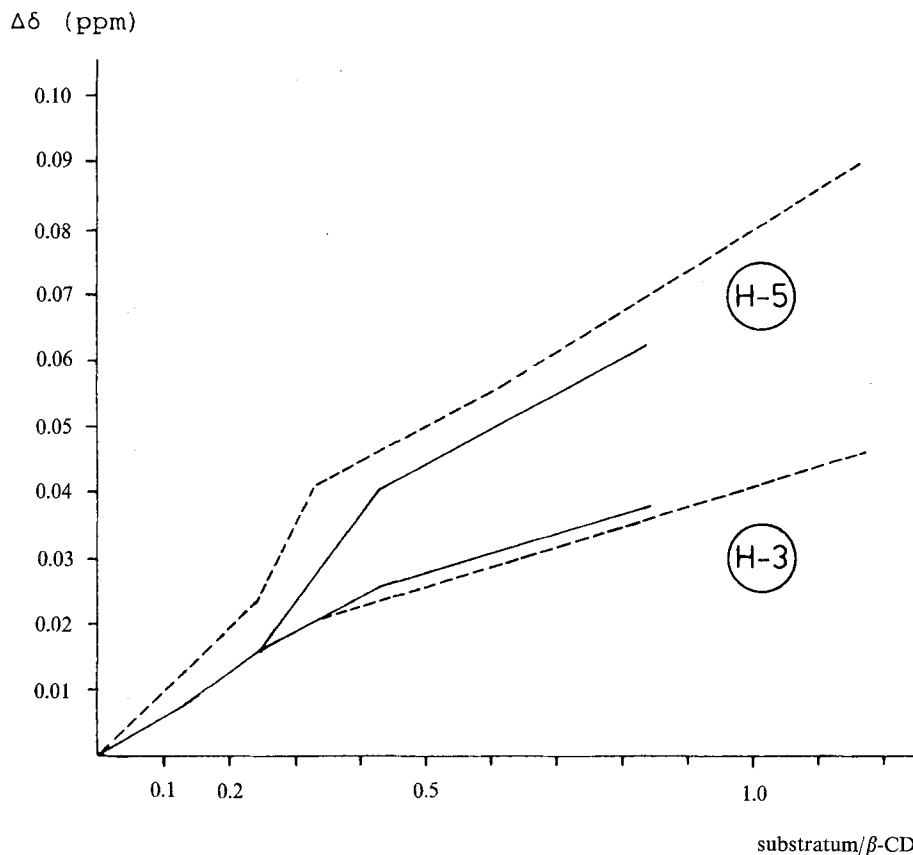


Fig. 1. Ephedrine and nitrosoephedrine induced shifts ($\Delta\delta$) of H-3 and H-5 protons of β -CD. — ephedrine ---- nitrosoephedrine.

and 1 : 1, respectively. The shift of H-1 was fixed with $\Delta\delta = 0$ ppm. The influence of the two included compounds on H-3 is very similar. But H-5 is shifted somewhat more by NEPH than by ephedrine. This indicates a greater stability of the β -CD-NEPH adduct.

The higher stability values of the NEPH inclusion compound and different solubilities can explain the catalytic nitrosation effect of the CDs.

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