Journal of Inclusion Phenomena **5** (1987), 625–629.

Nitrosation of Mefenorex in the Presence of Cyclodextrins★

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(Received: October 17 1986)

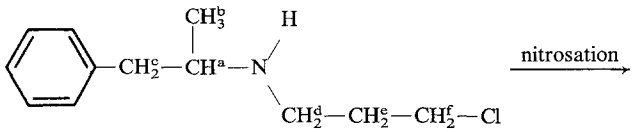
Abstract. β- and γ-Cyclodextrin (CD) and heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) form soluble inclusion compounds with mefenorex (MEF); with α-CD a partial inclusion occurs. No solid inclusion compound could be obtained with the four CDs. β-, γ-CD and DIMEB, but not α-CD, enhance the nitrosation rate of MEF if the nitrosation assay procedure (NAP test) is applied. During this reaction with β- and γ-CD, solid inclusion compounds of the CDs and nitrosomefenorex (NMEF) precipitate.

Key words: Nitrosation reactions: α-, β-, γ-cyclodextrin, dimethyl-β-cyclodextrin; mefenorex; nitrosomefenorex.

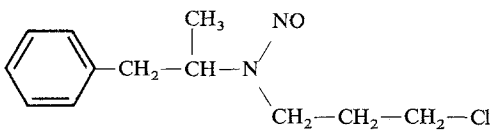
1. Introduction

The *in vitro* nitrosation rate of nitrosatable drugs can be influenced differently by α-, β- and γ-cyclodextrin (CD) and by heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB). The reaction rates of the fast nitrosatable piperazine, ethambutol and cimetidine are not influenced by α-, β- and γ-CD [1]. But, β-, γ-CD and DIMEB catalyze significantly the nitrosation of the slower nitrosatable ephedrine [2] and fencamfamine [1]. With these two drugs the formation of solid inclusion compounds of β-CD and nitrosoephedrine [2] and of γ-CD and nitrosofencamfamine [1] has been observed.

It is the purpose of this paper to examine whether the *in vitro* nitrosation of the secondary amine mefenorex (MEF), a potent anorectic, to *N*-nitrosomefenorex (NMEF) can be enhanced by CDs.



Mefenorex (MEF)



N-Nitrosomefenorex (NMEF)

★ Part of the Ph.D. thesis of V. Wedelich, Freie Universität Berlin, 1985.
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2. Materials and Methods

α-CD: Aldrich Europe, Beerse; β-CD: Chinoin Co., Budapest; γ-CD: Nihon Shokuhin Kako Co., Tokyo. DIMEB: Prepared according to Szejtli *et al.* [3]; Fp. 311 °C. The NMR spectrum corresponds to the specifications given by Casu *et al.* [4]. Mefenorex hydrochloricum: Homburg Co., Frankfurt/M.

The nitrosation procedure was performed with the nitrosation assay procedure (NAP test) as described before [1]. Solution I: mefenorex hydrochloricum 2.7301 g, HCl (37%) 0.5 ml, water to 500 ml; solution II: sodium nitrite 3.036 g to 100 ml water.

Inclusion compounds of β-CD or γ-CD and nitrosomefenorex: A solution of 50.0 mg β-CD or 57.1 mg γ-CD, respectively, in 2.0 ml solution I (37 °C) are mixed vigorously with 200 µl solution II (37 °C) in a small screw-topped 4.5-ml plastic tube. After 30 min the precipitate is centrifuged at 3000 rpm for 3 min. After decantation of the supernatant fluid the open plastic tubes were vacuum dried over calcium chloride at room temperature for 48 h. Yield: 30 mg white β-CD adduct, 40 mg γ-CD adduct.

^{1}H-NMR spectra were recorded on a 250 MHz spectrometer, WM 250 (Bruker Co., Karlsruhe) using 3-trimethylsilylpropionic acid-d_4-sodium as internal standard.

3. Results

3.1. FORMATION OF INCLUSION COMPOUNDS

The formation of CD-inclusion compounds with MEF and the formation of a nitroso-compound from MEF has not been reported up to now. It was not possible to obtain a solid inclusion compound with α-, β- and γ-CD and DIMEB, respectively.

But all four CDs form soluble inclusion compounds with MEF, which could be proved by ^{1}H-NMR. Table I shows the shifts of the CD protons in the presence of equimolar amounts of MEF and CDs. A shift of the protons H–3 and H–5, which are located in the inner part of the CD ring, indicates inclusion formation.

Table I. Chemical shifts ($\Delta\delta$) of the protons of α-, γ-CD and DIMEB in the presence of mefenorex.

$\Delta\delta$ (ppm) = $\delta_{\text{mefenorex + CD}} - \delta_{\text{CD}}$

CD-proton	α-CD	γ-CD	DIMEB
H–1	0.0179	0.0107	0.0194
H–2	0.0237	0.0170	0.0290
H–3	−0.0251	−0.0022	−0.0757
H–4	0.0165	0.0177	0.0253
H–5	0.0139	−0.0090	−0.0172
H–6	0.0084	0.0044	−0.1078

MEF + α-CD: Only H–3 is shifted to lower ppm values. This leads us to assume a partial inclusion of the aromatic moiety in the side *a* of the α-CD molecule (Fig. 1).

MEF + β-CD: The sift of the protons to a higher field is so pronounced that an overlapping with the MEF protons occurs. Therefore, no NMR data could be obtained, but an inclusion of the MEF molecule is obvious from the spectrum.

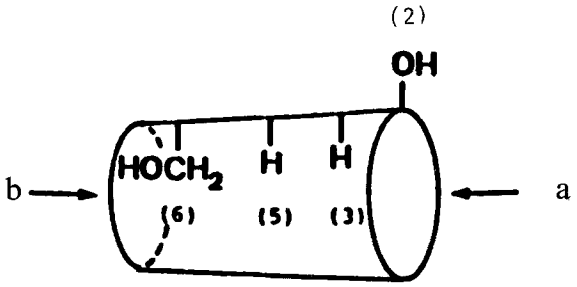

Fig. 1. Scheme of the cyclodextrin ring.

MEF + γ-CD: An inclusion of MEF exists. But, the stability of the inclusion compound should be low because of the insignificant influence of the guest molecule on H–3 and H–5.

MEF + DIMEB: The strong shift of H–3 and H–6 which is located at the rim of the closer b side (Fig. 1) indicates a very stable inclusion compound.

3.2. NITROSATION REACTIONS

MEF can form a nitroso-compound in a relatively slow reaction if nitrosated with the nitrosation assay procedure (Fig. 2). The existence of a maximum value after more than 60 minutes proves that a faster decomposition reaction superimposes the nitrosation process.

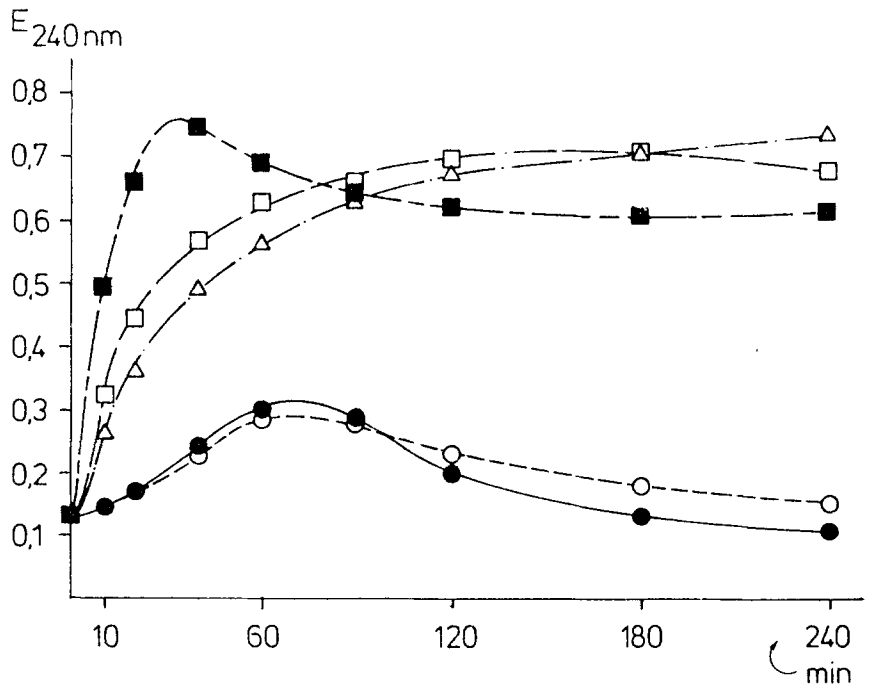

Fig. 2. *In vitro* nitrosation of mefenorex in the presence of cyclodextrins; ●——●, without CD; ○------○, with α-CD; □---□, with β-CD; △-·-·-△, with γ-CD; ■-----■, with DIMEB.

α-CD does not influence the reaction rate of the nitrosation process. β- and γ-CD and DIMEB increase the nitrosation considerably. DIMEB has the greatest effect as is proved by the early maximum. Whereas the reaction product with γ-CD does not show any instability, in the presence of β-CD a decomposition process can be observed.

In all reaction mixtures with β- or γ-CD, a precipitate is formed after about 10 minutes. Compared to the results with ephedrine [2] and fencamfamine [1], it is assumed that these precipitates are inclusion compounds of CDs and NMEF.

The reaction curves which were obtained in the presence of β-, γ-CD and DIMEB were significantly different from each other as well to the reaction curve without CD ($p < 0.001$), except the 90 min (β-/γ-CD/DIMEB), 120 and 180 min (β-/γ-CD) values. The difference between the curves with α-CD and without CD is significantly different after 180 minutes.

3.3. INCLUSION COMPOUNDS OF β- AND γ-CD AND NITROSOMEFENOREX

To demonstrate the composition of the precipitates, the ^{1}H-NMR spectra of the following samples were examined:

(A) β-CD or γ-CD, respectively
(B) Equimolar mixtures of MEF and β-CD or γ-CD
(C) The Precipitates obtained from the reaction mixtures

The unstable NMEF was not obtainable as a pure compound. The spectra of B and C contained the signals of the CD protons. Comparison of B and C showed all signals of the protons of MEF, but with somewhat different positions. The following $\Delta\delta$ values ($\delta_{\text{precipitate}} - \delta_{\text{MEF}}$) were obtained:

a: + 2.2 ppm, b: + 0.3 ppm, c: ± 0 ppm, e: − 0.5 ppm,
d and f: overlapped by CD signals, aromatic ring: ± 0 ppm.

The shift of the signals of protons a and b to higher ppm values is explained by the electron attracting effect of the neighbouring nitroso group. Protons e show the opposite effect. Probably the nitroso group compensates the electronegative effect of the chloro atom. From the integration curves of the spectra the molar ratio between NMEF and β-CD was 0.8 : 1.0, between NMEF and γ-CD 1.1 : 1.0. This indicates that the precipitates are inclusion compounds of NMEF and β-CD or γ-CD, respectively.

The reason for the increased activity is especially seen in the different solubilities and stabilities of the inclusion compounds formed. Because of the reduced polarity of NMEF compared to MEF, the tendency to interact with β- or γ-CD should be greater for NMEF. Additionally, the solubility product of the inclusion compounds with NMEF should be smaller than that with MEF. The precipitation results in a change of the reaction equilibrium [2]. This favors an increased reaction rate of the nitrosation process.

The high reactivity of MEF in the presence of CDs if the NAP test is applied leads us to assume that the nitrosatable amino group is located outside of the CD cavity if the complex has formed.

Acknowledgements

The authors are grateful to the Verband der Chemischen Industrie – Fonds der Chimie – for the support of this work, to Chinoin Co., Budapest (Prof. Szejtli), for gifts of β-cyclodextrin, and to Homburg Co. for gifts of mefenorex hydrochloricum.

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