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Journal of Chromatography A, 853 (1999) 455–460

JOURNAL OF CHROMATOGRAPHY A

Resolution of newly synthesized racemic dihydropyridines with different chiral selectors by means of capillary electrophoresis

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Abstract

The racemates of newly synthesized 4-aryl-1,4-dihydropyridine derivatives attracting interest in the treatment of coronary insufficience were resolved via the formation of diastereomeric salts. In order to check the quality of the preparative resolution, capillary electrophoresis using neutral cyclodextrins (CDs) was developed. In particular, the α-CD was found to be a powerful discriminator of the enantiomers. Additionally, taking amlodipine and nicardipine into consideration, a mechanism of the chiral recognition with α-CD could be proposed.

Keywords: Enantiomer separation; Pharmaceutical analysis; Dihydropyridines

1. Introduction

Calcium channel antagonists [1,2] of the 4-aryl-1,4-dihydropyridine type, such as nicardipine and amlodipine, are powerful vasodilatators with a minimal negative inotropic effect and, thus, effective for the treatment of hypertension [3,4]. In contrast, some 5-cyano- and 5-nitro-4-aryl-1,4-dihydropyridines are calcium channel activators showing a positive inotropic as well as a vasoconstrictive and, along with this, a hypertensive activity [5,6]. New nitrooxylated 5-cyano- and 5-nitro-4-aryl-1,4-dihydropyridine hybrid structures were synthesized to overcome the vasoconstriction by incorporation of an organic nitrate [7]. The resulting compounds should have positive inotropic, but no vasoconstrictive properties and, thus, be useful for the treatment of coronary insufficiency.

The activity of a chiral dihydropyridine depends sensitively on the configuration in position 4, which affords the synthesis of enantiomerically pure compounds. The intermediate dihydropyridine carboxylic acids **1–4** (Fig. 1) of the new hybrid molecules were used to resolve the racemic mixture by means of brucine [8]. From the reaction mixture, one diastereomeric salt crystallized spontaneously. Through extraction with hydrochloric acid and ethyl acetate, the enantiomerically pure carboxylic acids could be obtained [7].

The purpose of this study was to develop a method which is suitable to determine the enantiomeric excess (ee) of the resolved dihydropyridine acids. Cyclodextrin (CD)-modified capillary electrophoresis (CE) has been proved to be extremely effective in the resolution of racemic drugs [9–13], and can be

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00566-X

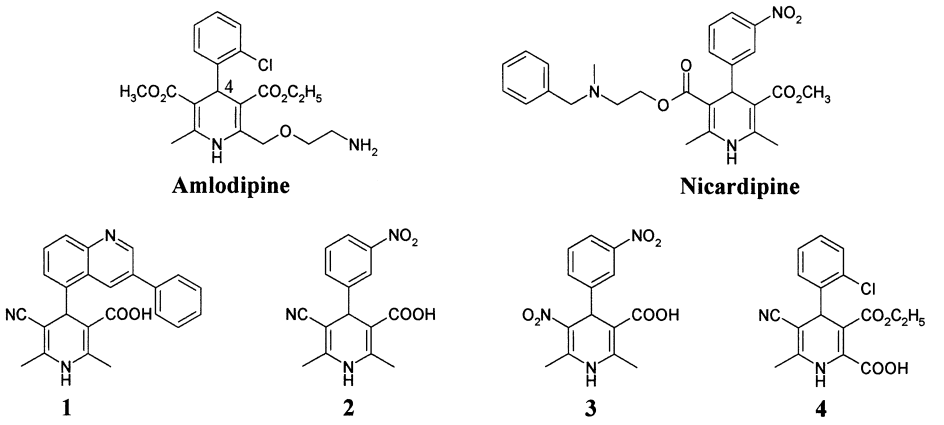

Fig. 1. Structures of the 1,4-dihydropyridine compounds studied.

used for the evaluation of the ee [14–20]. The dihydropyridine compounds were subjected to CE using neutral cyclodextrins such as α-, β- and γ-CD [21–24]. For the sake of comparison, the structurally corresponding dihydropyridines in clinical practice, i.e., amlodipine and nicardipine (Fig. 1), were included in this study.

2. Experimental

2.1. Chemicals

The racemates of amlodipine besylate and nicardipine·HCl were provided by Pfizer (Karlsruhe, Germany) and Novartis Pharma (Wehr/Baden, Germany), respectively, the new dihydropyridines were synthesized according to Ref. [7]. α-, β- and γ-CDs were a gift from the Consortium für Elektrochemische Industrie (Munich, Germany). NaH_2PO_4, Na_2HPO_4, orthophosphoric acid, methanol, acetonitrile and tetrahydrofuran were purchased from Merck (Darmstadt, Germany).

Phosphate buffer, pH 3 was prepared by mixing appropriate concentrations of H_3PO_4 and NaH_2PO_4 solution. Phosphate buffers, pH 4.5–8.0 were prepared by mixing appropriate concentrations of NaH_2PO_4 and Na_2HPO_4 solutions. The samples subjected to the CE were dissolved in methanol (conc. ≈1 mg/ml) and diluted with deionized water (1:20). All solutions were prepared with deionized water and filtered through a 0.45-μm single syringe (Schleicher und Schüll, Germany).

2.2. Capillary electrophoresis

All experiments were performed on a Beckman P/ACE 5500 system (Fullerton, CA, USA) using a fused-silica capillary of 47 cm (detection length 40 cm)×75 μm I.D. Samples were loaded by 5 s of pressure injection and separated at 25°C using a constant voltage of 6 to 10 kV. For detection, a diode array detector was used within 190 to 300 nm.

In order to optimize the separation conditions of each newly synthesized dihydropyridine acid (**1–4**), the neutral cyclodextrin derivatives were dissolved in 50 m*M* phosphate buffers of pH 6 and 8 and the following parameters were varied: the CD concentrations in a range from 0.0015 to 0.018 *M*, the organic modifiers, acetonitrile, tetrahydrofuran and methanol, in a range of 0 to 15%. In order to optimize the resolution of amlodipine and nicardipine, these compounds were measured in 50 m*M* phosphate buffers of pH 3, 4.5 and 6 and the concentration of the various CDs varied in a range of 0.0015 to 0.018 *M*.

The capillary was conditioned for 20 min with 0.1 *M* NaOH and 10 min with water. Additionally, the capillary was washed for 2 min with 0.1 *M* NaOH, 1 min with water and 2 min with the running buffer before each run.

3. Results and discussion

3.1. CE studies of the newly synthesized dihydropyridine carboxylic acids

In order to optimize the resolution conditions for the enantiomers of the newly synthesized dihydropyridine acids **1–4**, the three neutral CDs, α-, β- and γ-CD, were studied at neutral to basic pH values in order to ensure that the acidic dihydropyridines were negatively charged. Thus, the electrophoretic mobility of the analyte is directed to the anode. The electroosmotic flow (EOF), caused by the deprotonation of the silanol groups of the capillary wall at these pH values, is strong enough to carry the analytes to the cathode [9,25]. For each CD type, the CD concentration was varied (cf. [25,26]) and at the CD concentration showing the best resolution, organic modifiers in varying amounts (cf. [27]) were added to the background electrolyte.

3.1.1. CD variations

With α-CD, the racemates of the dihydropyridines **1–3** could be increasingly resolved with increasing CD concentration at pH 8 (see Fig. 2A). The isomers of compound **3** already showed a baseline separation at a concentration as low as 0.003 *M* α-CD. In the case of the compounds **1** and **2**, a CD concentration

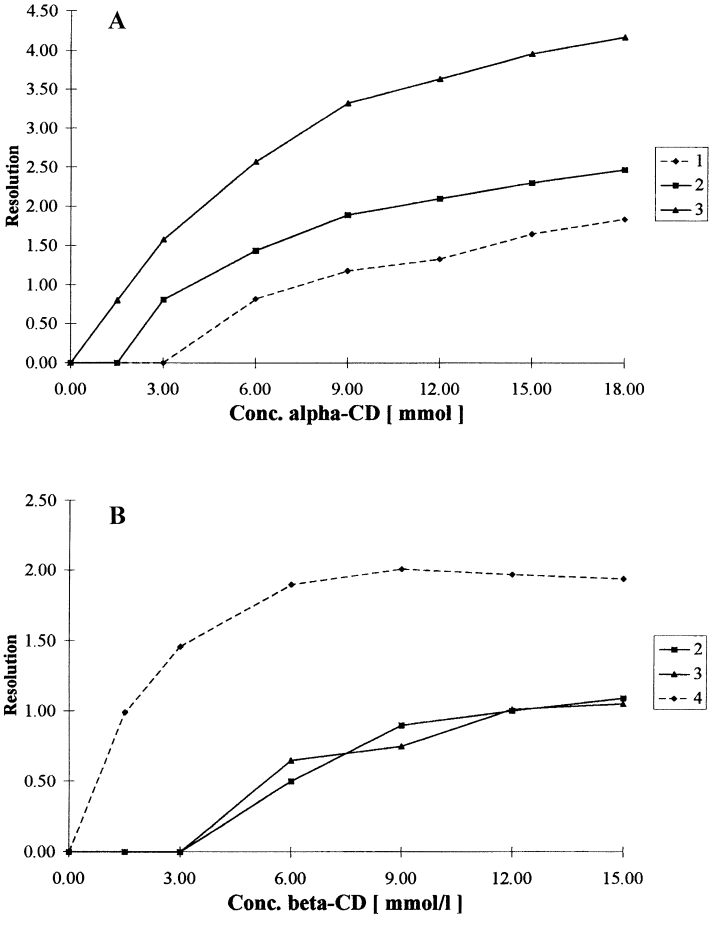

Fig. 2. (A) Resolution of the compounds **1–3** using different concentrations of α-CD in 50 m*M* phosphate buffer, pH 8 at a constant voltage of 6 kV. (B) Resolution of the compounds **2–4** using different concentrations of β-CD in 50 m*M* phosphate buffer, pH 8 at a constant voltage of 6 kV.

of at least 0.015 *M* is necessary to completely resolve the enantiomers. Compound **4** having the carboxylic group in position 2 did not even show a shoulder in the peak.

With β-CD, the enantiomers of the dihydropyridines **2–4** could be separated, but even at the highest CD concentration, the enantiomers are hardly baseline separated (see Fig. 2B). Thus, the resolution power of β-CD is much poorer than of α-CD. Nevertheless, β-CD is the only CD which is able to resolve the enantiomers of **4**.

With γ-CD, only the enantiomers of compound **3** could be resolved with a poor resolution value of 1.16 at pH 8 and 1.59 at pH 6.

In Table 1, the best resolution values obtained are depicted. At this point, it can be summarized that a complete resolution of the dihydropyridines **1–3** can be achieved with α-CD; compound **4** can be only resolved with β-CD.

3.1.2. Organic modifier

In order to check whether an organic modifier is able to enhance the already obtained resolutions, methanol, tetrahydrofuran and acetonitrile were added to the background electrolyte in increasing amounts [28,29]. In these experiments, the CD concentration amounts to 0.018 *M*. All modifiers decreased the resolution for all compounds with increasing concentration and lipophilicity of the modifier. E.g., the resolution of **2** completely collapsed at tetrahydrofuran concentrations less than 2% and acetonitrile concentration of 2 to 3%. In the case of methanol, a baseline resolution of the enantiomers of **2** could be still observed upon addition of 15% solvent. Qualitatively similar results were obtained for the compounds **1**, **3** and **4**. The effects might be caused by a competition between the analyte and the modifier for the cavity: the higher the amount of modifier, the smaller the portion of the analyte in the cavity and, along with that, the lower the resolution.

Interestingly, the addition of the organic modifier increased the migration time by about 50%. This might be caused by the decrease of the current upon addition of the organic solvents.

3.1.3. Determination of the ee

Using the optimized conditions displayed in Table 1, the ee for compounds **2–4** were determined. The samples studied were the best resolutions of the racemates obtained via the formation of diastereomeric salts. Since the resolution values were found to be in a range of 2 to 5, the enantiomeric excess could be determined with high precision, independent of the migration order. As can be seen from Fig. 3, the enantiomers of **2–4** show a very high enantiomeric purity: the ee values were found to be higher than 90%. It is worth mentioning that, considering the optical rotation, the dihydropyridine **2** had a reverse migration order in comparison with the compounds **3** and **4**. The preparative resolution of compound **1** was not yet successful.

3.2. CE studies of amlodipine and nicardipine

Since amlodipine having an *o*-chlorosubstituted phenyl ring in position 4 and nicardipine having an *m*-nitrosubstituted phenyl ring are comparable to compound **4** and to the compounds **2** and **3**, respectively, they are included in this study. In order to find out whether the 4-phenyl ring participates in the complexation with the CD and, along with that, in the process of the chiral recognition, these dihydropyridines were checked under comparable conditions using the neutral CDs. However, the resolution of amlodipine and other chiral dihydropyridines in clinical practice with negatively charged CDs was already reported [30–33].

Table 1
Migration times (t_M) and resolution for the enantiomers of compound **1–4** in 50 m*M* phosphate buffer

Compound	CD additive	pH	Constant voltage (kV)	t_{M1} (min)	t_{M2} (min)	Resolution
1	18 m*M* α-CD	8	10	12.74	13.12	2.16
2	18 m*M* α-CD	8	10	9.91	10.19	2.73
3	18 m*M* α-CD	6	8	20.70	22.59	5.83
4	9 m*M* β-CD	8	8	13.97	14.44	2.24

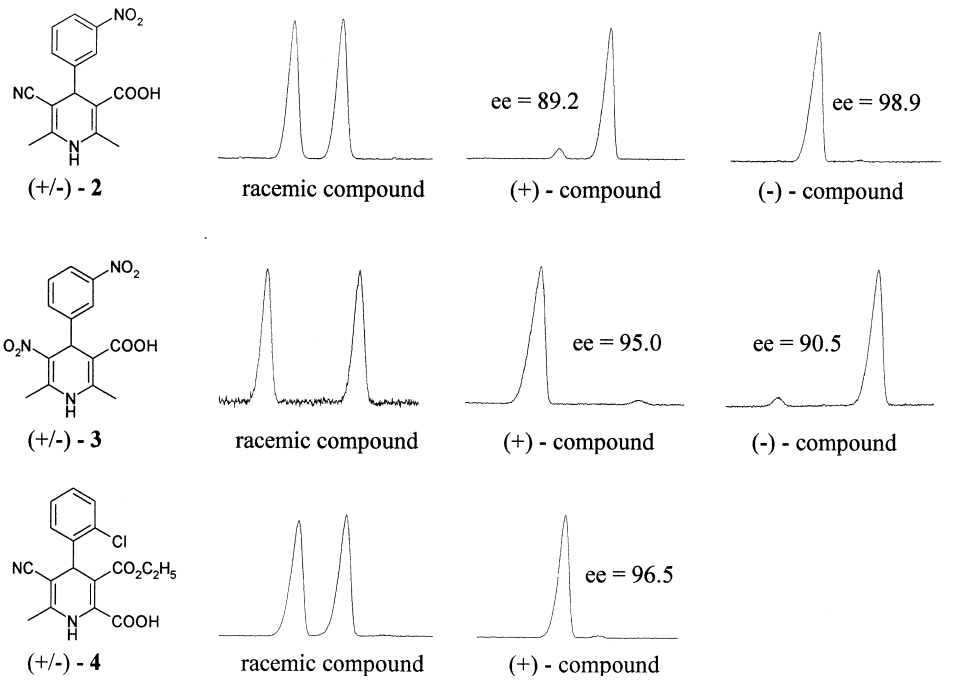

Fig. 3. Determination of the enantiomeric excess (ee) for the compounds **2–4**.

A baseline separation of the enantiomers of amlodipine could only be achieved with α-CD (18 m*M* CD, pH 3). Interestingly, the resolution observed in this study appeared to be higher than the resolution obtained by Owens et al. [31] under similar conditions. For nicardipine, no resolution with either CD type was achieved. With γ-CD, a shoulder could be only observed. However, preliminary investigations revealed a rather good resolution for amlodipine (cf. [31,32]) and nicardipine using the negatively charged sulfobutylether β-CD, introduced by Tait et al. [34] and *heptakis*(2,3-*O*-acetyl-6-sulfato)β-CD, developed by Vincent et al. [35].

4. Discussion

With the exception of nicardipine, the enantiomers of all dihydropyridine derivatives studied could be well resolved by using neutral CDs. Especially the α-CD characterized by the smallest cavity of all CDs is able to discriminate between the enantiomers of the dihydropyridines.

In order to explain the mechanism of the chiral recognition, compounds with corresponding moieties have to be compared under identical CE conditions. Especially, the results obtained with α-CD will be discussed. The resolution of the dihydropyridines having an *m*-nitrosubstituted phenyl ring in position 4, i.e., **2**, **3** and nicardipine, is found to be rather different: whereas the enantiomers of **2** and **3** are well resolved, R_s=2.73 and 5.83, respectively, the racemate of nicardipine did not show any sign of resolution. Amlodipine and the dihydropyridine **4** both characterized by an *o*-chlorosubstituted aromatic ring show similar differences in the resolution: the racemate of amlodipine could be resolved whereas the enantiomers of **4** could not be separated using α-CD. In addition, the racemate of compound **1** having a quinoline ring in position **4** which is known not to fit into the cavity of α-CD [36] is well resolved. These findings may indicate that the phenyl ring in position 4 does not participate in the process of the chiral recognition. In turn, the dihydropyridine ring should be involved in the complexation which determines the discrimination between the enantiomers. Thus, the substitution pattern of the dihydropyridine ring should sensitively influence the res-

olution. The comparison of compounds with an identical substitution on the dihydropyridines, such as **1** and **2**, revealed almost the same extent of resolution, 2.16 versus 2.73. Comparing in the next step compounds with slightly different substitution on the dihydropyridines, but an identical aryl moiety, i.e., **2** and **3**, showed rather different values of resolution, 2.73 versus 5.83. Additionally, **2** and **3** were found to have a reverse migration order. These results support the hypothesis that the dihydropyridine ring is responsible for the chiral recognition with α-CD. It is likely that inclusion complexes are formed.

Due to the poor resolution power of β-CD in comparison with α-CD the results achieved with β-CD are difficult to discuss. Nuclear magnetic resonance experiments are under progress to gain more insight into the mechanism of chiral recognition of the different cyclodextrins.

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