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Capillary zone electrophoretic separation of epimeric *N*-oxides of morphinane alkaloids

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Abstract

Epimers of *N*-oxides of morphine, codeine, thebaine, pseudomorphine and 2,2′-biscodeine were separated by capillary zone electrophoresis in the presence of cyclodextrins in bare silica or poly(vinyl alcohol)-coated capillaries in a background electrolyte with Tris–phosphate buffer, pH 2.8; diastereomers of thebaine *N*-oxide were separated also without cyclodextrins. γ-Cyclodextrin caused the highest resolution of epimers of morphine and codeine *N*-oxides, while separation of mono- and di-*N*-oxides of pseudomorphine and 2,2′-biscodeine without stereodifferentiation was best in the presence of 2,6-di-*O*-methyl-β-cyclodextrin. However, addition of the former cyclodextrin resulted also in separation of diastereomers of bismorphinane *N*-oxides.

Keywords: Diastereomer separation; Alkaloids; Cyclodextrins; Morphinane *N*-oxides

1. Introduction

Morphinane alkaloids having a free phenolic group in their structure are in aqueous solution oxidized by oxygen of air into particular 2,2′-dimers and *N*-oxides [1,2]; derivatives with the C-3 hydroxyl bonded in an ether or ester grouping gave the *N*-oxides only. This oxidation is a problem mainly in preparation and stability of injections, however, morphine *N*-oxide was detected also in opium [3], in urine of the experimental animals after administration of morphine [4], or it was prepared by incubation of morphine with a hepatic microsomal drug oxidase [5]. *N*-Oxides are prepared on a preparative scale by oxidation of an alkaloid with H_2O_2 or peroxoacids; a pair of diastereoisomers is generated by this reaction, because oxygen attached to N-17 can occupy equatorial or axial position. Morphine (**1**), codeine (**2**) and thebaine (**3**) oxidized with H_2O_2 afforded the mixture of *N*-oxides, while reaction with *m*-chloroperbenzoic acid gave only one diastereoisomer of morphine and codeine in contrast to thebaine (**3**) which also under these conditions afforded two epimers [6]. Nuclear magnetic resonance (NMR) experiments revealed that the major epimer represents the *N*-oxide with an axially attached oxygen (**4a**, **5a**, **6a**) and the minor one has a N–O bond in an equatorial position (**4e**, **5e**, **6e**) [7].

Recently, a separation of epimeric pavine alkaloid *N*-oxides from *Thalictrum simplex* L. by reversed-phase high-performance liquid chromatography (HPLC) was reported [8] but we did not find any method for separation of epimers of morphinane *N*-oxides. Separation of these compounds by capillary zone electrophoresis (CZE) is described in this paper.

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2. Experimental

2.1. Capillary electrophoresis

A HP ^{3D}CE apparatus (Hewlett-Packard, Waldbronn, Germany) with diode array detection (DAD) (190–600 nm) was used for analysis. Detection was at a fixed UV wavelength of 215 nm with data processed on a HP ChemStation. Capillaries were untreated fused-silica capillary tubes of 48.5 and 64.5 cm (effective length 40 and 56 cm, respectively)×0.05 mm I.D. and an extended light path (bubble factor 3) as well as a 64.5 cm×0.05 mm capillary tube coated with poly(vinyl alcohol) (PVA). Prior to use, the bare silica capillary was rinsed with 1 *M* NaOH (15 min), distilled water (10 min) and the appropriate background electrolyte (BGE) (5 min); the PVA-coated capillary was rinsed with 20 m*M* H_3PO_4 (20 min), distilled water (10 min) and the buffer (5 min). Between analyses both capillaries were flushed with 10 m*M* H_3PO_4 (2 min), distilled water (1 min) and buffer solution (3 min). Samples were kept at laboratory temperature in the autosampler and pressure injected at 5 kPa for 2 s. Resolution of peaks was calculated according to equation $R_s=2(t_2-t_1)/(w_1+w_2)$ where t=migration time and w=baseline peak width.

2.2. Drugs and chemicals

Morphine (**1**), codeine (**2**) and thebaine (**3**) were from Slovakofarma (Hlohovec, Slovak Republic), and pseudomorphine (**7**) and 2,2′-biscodeine (**8**) were synthesized [9] and isolated in our laboratory, respectively. Major epimers of *N*-oxides were synthesized according to Ref. [6]. All prepared compounds were characterized by spectral data.

β-Cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), γ-cyclodextrin (γ-CD), 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were from Fluka, Buchs, Switzerland; heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (D-β-CD was from Sigma, St. Louis, MO, USA and carboxylated-β-cyclodextrin (CY-β-CD) was purchased from Cyclolab (Budapest, Hungary). Pyridoxol hydrochloride was used as a reference substance and all other chemicals were from Fluka.

2.3. Preparation of solutions of alkaloid N-oxides

Alkaloid (75 mg) dissolved in CH_3OH (10 ml) was mixed with H_2O_2 (2 ml), or *m*-chloroperbenzoic acid (40 mg) was added and the solution was heated at 45°C for 5 min. The reaction mixture was cooled, and 1 ml of this solution was transferred to a volumetric flask (10 ml), where HCl (0.5 *M*, 1 ml) and a solution of the reference substance (pyridoxol hydrochloride, 30 mg/50 ml of water, 1 ml) were added and the volume made up with distilled water. Solution before injection was filtered through a 0.20-μm membrane filter.

Contrary to published data [6] we found that oxidation of morphine and codeine with *m*-chloroperbenzoic acid also afforded a mixture of epimers; the ratio of epimers obtained with H_2O_2 was 10:1, with *m*-chloroperbenzoic 20:1; ratio of thebaine *N*-oxide epimers prepared with both oxidants was about 5.5:4.5 in favor of the (17*R*)-epimer **6a**.

2.4. Reduction of N-oxides

The reaction mixture (5 ml) after oxidation with *m*-chloroperbenzoic acid was combined with a solution of Na_2SO_3 (50 mg/25 ml of water), then solution was acidified with aqueous HCl (0.5 *M*, 1 ml) and vigorously mixed at ambient temperature; 2 ml of this solution was pipetted into a volumetric flask (10 ml), HCl (0.5 *M*, 1 ml), marker solution (1 ml) added and the volume was completed with distilled water.

3. Results and discussion

Alkaloid *N*-oxides have lower mobility in acidic BGEs and separation of both groups by CZE is quite satisfactory. However, morphinane alkaloids oxidized with peroxides form two epimers, with oxygen oriented equatorially (17*S*, **4e**) or axially (17*R*, **4a**). Separation of these epimers in a 40 cm long bare silica capillary and plain BGE was observed only with thebaine *N*-oxides, however, addition of cyclodextrins significantly improved resolution of **6a** and **6e** (Fig. 1) and D-β-CD was the best selector for resolution. Epimers of *N*-oxides of other morphinane alkaloids were separated only in presence of chiral

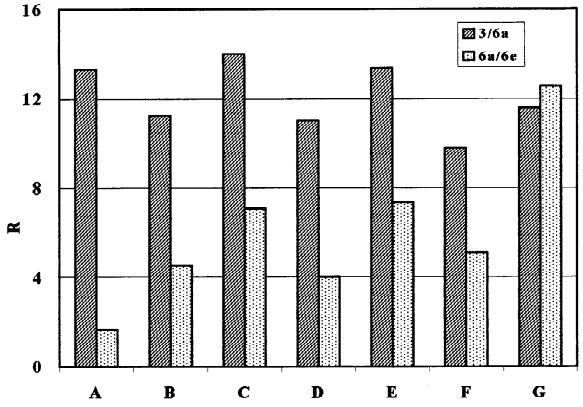

Fig. 1. Resolution of peaks of thebaine (**3**) and epimers of its *N*-oxide **6a** and **6e** in the presence of cyclodextrins. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C, 5 m*M* CD. (A) Without CD, (B) β-CD, (C) γ-CD, (D) HP-β-CD, (E) HP-γ-CD, (F) CY-β-CD, (G) D-β-CD.

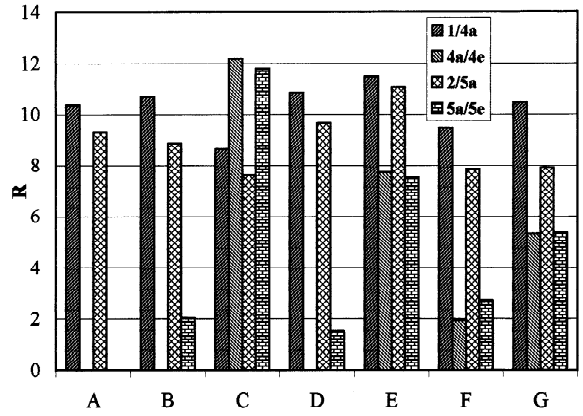

Fig. 2. Resolution of peaks of morphine (**1**), codeine (**2**) and epimers of their *N*-oxides **4a** and **4e** and **5a**, **5e** in the presence of cyclodextrins. Conditions as in Fig. 1.

selectors (Table 1, Figs. 2 and 3). The diameter of the cyclodextrin cavity was an important factor for resolution of epimers of morphine and codeine *N*-oxides (**4a**, **4e** and **5a**, **5e**, respectively) and to a lesser extent also of thebaine *N*-oxides (**6a**, **6e**): none or lower resolution in BGE with β-CD, very good separation in presence of γ-CD. Hydroxypropylation of cyclodextrins lowered the discrimination of epimers, as is best documented on codeine *N*-oxides (Fig. 2, β-CD/HP-β-CD, γ-CD/HP-γ-CD). Higher resolution in series of β-CD proved addition of

Table 1
Relative migration times of studied compounds with pyridoxol as a reference compound (REF)

No.	Cyclodextrin						
	No CD	β-CD	γ-CD	HP-β-CD	HP-γ-CD	CY-β-CD	D-β-CD
1	1.18	1.20	1.28	1.19	1.22	1.23	1.21
2	1.16	1.20	1.26	1.20	1.21	1.28	1.22
3	1.16	1.24	1.23	1.21	1.20	1.43	1.24
4a	1.30	1.32	1.40	1.31	1.37	1.38	1.31
4e	1.30	1.32	1.56	1.31	1.46	1.46	1.33
5a	1.29	1.30	1.38	1.31	1.34	1.43	1.32
5e	1.29	1.33	1.56	1.34	1.43	1.53	1.35
6a	1.32	1.39	1.41	1.35	1.40	1.63	1.3
6e	1.34	1.46	1.50	1.41	1.49	1.87	1.45
7	1.02	1.02	1.36	1.02	1.15	1.02	1.02
8	1.02	1.03	1.37	1.03	1.17	1.03	1.03
9a	1.08	1.09	1.40	1.08	1.22	1.12	1.08
9e	1.08	1.09	1.48	1.08	1.25	1.12	1.08
10aa	1.14	1.15	1.47	1.15	1.29	1.21	1.14
10ae	1.14	1.15	1.57	1.15	1.33	1.21	1.14
11a	1.09	1.09	1.43	1.09	1.21	1.11	1.09
11e	1.09	1.09	1.49	1.09	1.25	1.11	1.09
12aa	1.16	1.16	1.50	1.15	1.27	1.22	1.15
12ae	1.16	1.16	1.57	1.15	1.33	1.22	1.15
REF/min	3.82	3.82	3.82	3.91	3.91	3.82	3.82

Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate buffer, pH 2.83, 30 kV, 25°C, 5 m*M* CD.

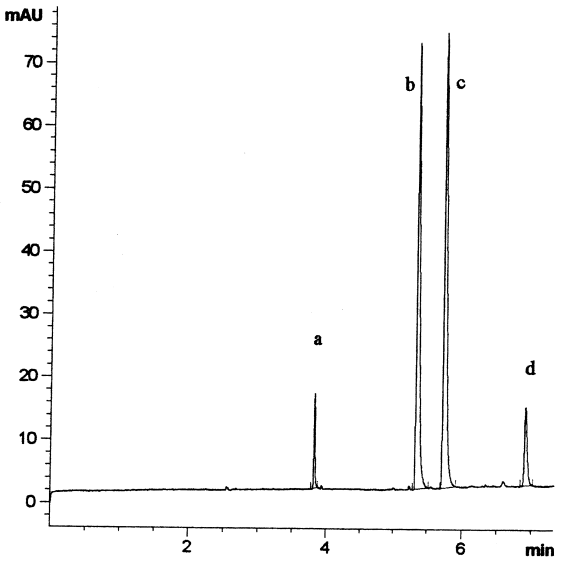

Fig. 3. Electropherogram of a mixture of codeine oxidation. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C, 5 m*M* γ-CD; pressure injection 10 kPa s^{-1}. Peaks: a=pyridoxol (0.3 m*M*), b=codeine (**2**, 1.08 m*M*), c=ax-*N*-oxide **5a** (1.14 m*M*), d=eq-*N*-oxide **5e** (0.16 m*M*).

CY-β-CD. At a low pH (2.8) BGE it behaved as a quasi stationary phase forming hydrogen bonds with separated compounds, but it caused unacceptable broadening of peaks at pH above 4. Methoxylation of β-CD causing enhancement of hydrophobicity of

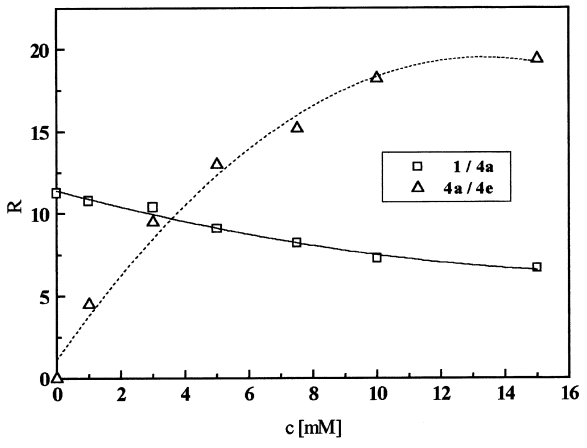

Fig. 4. Resolution of peaks of morphine (**1**) and its epimeric *N*-oxides **4a**, **4e** at increasing concentration of γ-CD. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C.

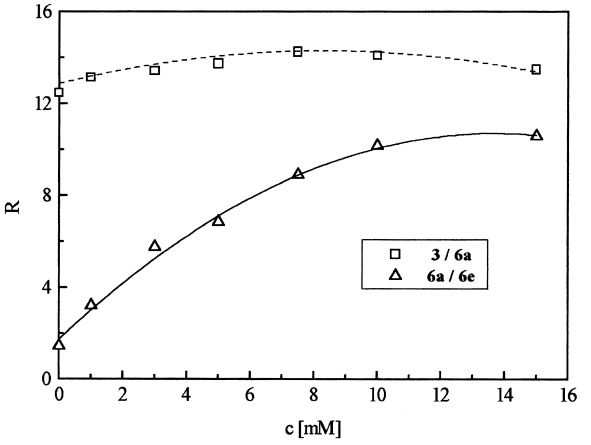

Fig. 5. Resolution of peaks of thebaine (**3**) and its epimeric *N*-oxides **6a**, **6e** at increasing concentration of γ-CD. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C.

selector cavity improved resolution also with morphine *N*-oxides (**3a**, **3e**) but only in comparison with other derivatives of β-CD. The (17*S*)-epimers (equatorially oriented oxygen) in all the studied *N*-oxides had lower mobility than their counterparts. Resolution of peaks of studied epimers was dependent on cyclodextrin concentration: while the resolution of peaks of parent alkaloid and (17*R*)-*N*-oxide was

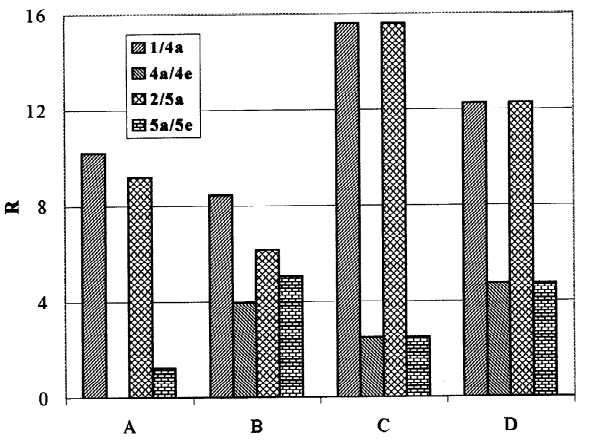

Fig. 6. Resolution of peaks of morphine (**1**), codeine (**2**) and epimers of their *N*-oxides **4a**, **4e** and **5a**, **5e**. Capillary: 64.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C. (A) Bare silica capillary, BGE without CD; (B) bare silica capillary, BGE with 10 m*M* D-β-CD; (C) PVA-coated capillary, without CD; (D) PVA-coated capillary, BGE with 10 m*M* β-CD.

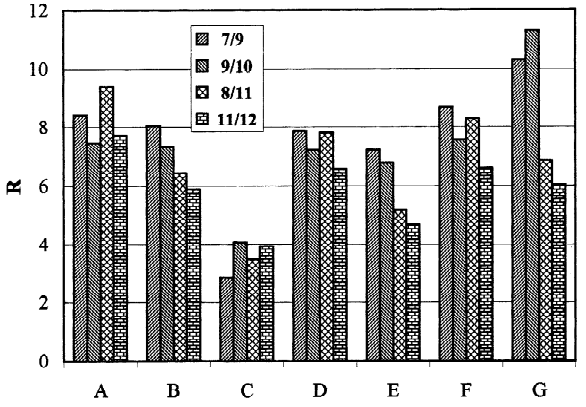

Fig. 7. Resolution of peaks of pseudomorphine (**7**), 2,2′-biscodeine (**8**) and their mono- and di-*N*-oxides **9**, **10** and **11**, **12**, respectively in the presence of cyclodextrins. Conditions as in Fig. 1. (Resolution of peaks of major epimers in C and E).

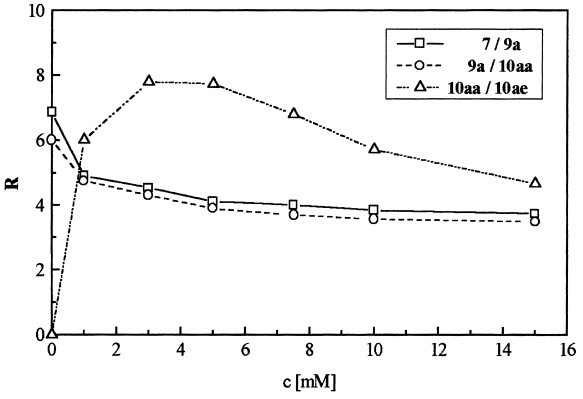

Fig. 9. Resolution of peaks of pseudomorphine (**7**), its *N*-oxide **9a** and epimeric di-*N*-oxides **10aa** and **10ae** at increasing concentration of γ-CD. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phosphate, pH 2.8, 30 kV, 25°C.

affected only to a small extent, separation of both epimers was substantially enhanced by higher concentration of selector (Figs. 4 and 5).

When the longer capillary was used (56 cm) under the same conditions, resolution of codeine *N*-oxides (**5a**, **5e**), but not of morphine *N*-oxides (**4a**, **4e**) also without cyclodextrin in BGE was observed, however, sufficient separation of these *N*-oxides was achieved in a capillary coated with PVA (Fig. 6). We suppose that this latter effect was result of a lower adsorption of solutes on the capillary wall.

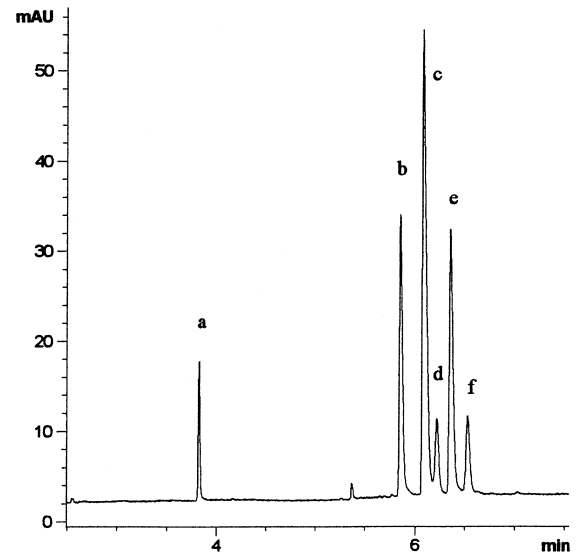

Fig. 8. Electropherogram of reaction mixture of 2,2′-biscodeine oxidation with H_2O_2. Capillary: 48.5 cm×0.05 mm, 100 m*M* Tris–phoshpate, pH 2.8, 30 kV, 25°C, 5 m*M* γ-CD; pressure injection 10 kPa s^{-1}. Peaks: a=pyridoxol (0.3 m*M*), b=2,2′-biscodeine (**8**, 0.16 m*M*), c=ax-*N*-oxide **11a** (0.56 m*M*), d=eq-*N*-oxide **11e** (0.09 m*M*), e=ax,ax-di-*N*-oxide **12aa** (0.29 m*M*), f=ax,eq-di-*N*-oxide **12ae** (0.08 m*M*).

Pseudomorphine (**7**) and 2,2′-biscodeine (**8**) are stepwise oxidized into mono- and di-*N*-oxides **9**, **10** and **11**, **12**, respectively which are readily converted into parent compounds by reduction with Na_2SO_3. Separation of these *N*-oxides, but not their epimers, was sufficient also in BGE without cyclodextrins. Addition of selectors affected resolution of peaks of mono- and di-*N*-oxides, the lowest was in presence of γ-CD (Fig. 7); however, separation of particular axial and equatorial stereoisomers of dimeric *N*-oxides was observed in BGEs with γ-CD (Fig. 8) and HP-γ-CD. Resolution of peaks of pseudomorphine ax,ax-di-*N*-oxide (**10aa**) and ax,eq-di-*N*-oxides (**10ae**) was sensitive to γ-CD concentration, with a maximum at 4 m*M* of added selector (Fig. 9).

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