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Reversed determination of the formation constants of 1-allyl terguride with mandelic acid optical isomers using capillary electrophoresis

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Abstract

A method is presented for the accurate determination of equilibrium constants of complex formation between mandelic acid enantiomers and 1-allyl terguride. In earlier studies this ergot alkaloid has proven to be a potential chiral selector for racemic acidic compounds. In these studies, the formation constant was determined by measuring the effective mobility of some racemic acidic analytes at varying concentrations of ergot alkaloid. For these experiments, the assumption was made that the complex mobility was zero. In order to validate these data, the experimental set-up was reversed. In this new set-up, the cationic chiral selector is injected as sample, while the background electrolyte (BGE) contained either of the two optically pure mandelic acid enantiomers, in varying concentrations. For accurate determination of the effective mobility, tetrabutylammonium was used as a mobility reference and the ionic strength of the BGE was kept constant. By performing these experiments at two different pH values, it was possible to determine complex-formation constants, and chiral selectivity towards both the dissociated and the non-dissociated mandelic acid enantiomers. Results show that only the dissociated acid interacts selectively with the ergot alkaloid confirming our earlier results. In our earlier experiments we made the assumption that the non-dissociated acid does not interact with the ergot alkaloid. The experimental data obtained by the current method however, show that, although this interaction is not enantioselective, it cannot be neglected. Optically pure mandelic acid proved to be a suitable chiral selector for the separation of terguride enantiomers.

Keywords: Chiral selectors; Enantiomer separation; Allyl terguride; Mandelic acid; Organic acids

1. Introduction

Chiral separations have become an important application in capillary electrophoresis [1]. In order to obtain more insight into the separation of enantiomers, the separation mechanism is discussed in several studies [2–7]. These studies focus on the determination of equilibrium constants of complex formation between analytes and cyclodextrins (CDs) as chiral selector (CS). For this purpose, the CS is dissolved in the background electrolyte (BGE) and the mobility of the optical isomers is determined as a function of the cyclodextrin concentration.

In some cases, this method has drawbacks. If the chiral selector is very high-priced, the determination of the formation constant can become very expensive, since not only the capillary but (in most cases) also the in- or outlet vial (or both) have to be filled with BGE containing CS. Only in the case where coated capillaries (or low pH BGEs) and neutral CSs

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are used, it is sufficient just to fill the capillary with the CS. Another obstacle can occur if the CS is a strong UV-absorbing compound. In case detection of the analytes is not possible on top of a high background absorption of the CS, it is necessary to fill only part of the capillary with BGE containing the CS [8,9]. In all these cases, the determination of formation constants can be troublesome, or even impossible.

Earlier, we studied the chiral separation mechanism of 1-allyl-(5*R*,8*S*,10*R*)-terguride (allyl-TER) for the separation of some racemic organic acids [10,11]. Allyl-TER is a strong UV-absorbing cation, and therefore (as outlined earlier) it was hard to determine the formation constants, without making some assumptions. In our earlier study [11], we assumed that only the dissociated acid interacted with the ergot alkaloid.

Recently, Lee and Lin [12] determined formation constants of cyclodextrins and some non-chiral compounds (salicylic acid and benzylamine) by injecting the cyclodextrins in a BGE containing different concentrations of these compounds. A similar approach was used in the current study. The formation constants were determined by measuring the mobility of the ergot alkaloid, injected as analyte, in BGEs consisting of varying concentrations of either L(+)- or D(−)-mandelic acid at different pH values. In this way, it was possible to determine both selectivity and formation constants for ionic and non-ionic complex formation [4,5].

2. Experimental

All experiments were performed on a P/ACE 2200 (Beckman, Fullerton, CA, USA) capillary electrophoresis apparatus. A fused-silica capillary with a total length of 568 mm (effective length 500 mm), and an internal diameter (I.D.) of 50 μm was used. A UV detector was applied at 230 nm. The capillary cartridge was thermostated at 25°C.

(+)-(5*R*,8*S*,10*R*)-1-Allyl-terguride was synthesized according to the procedure published elsewhere [13]. (+)- and (−)-Terguride [(+)- and (−)-TER] were a gift of the Academy of Sciences of the Czech Republic (Prague, Czech Republic). L(+)- and D(−)-mandelic acid were obtained from Fluka (Buchs, Switzerland). Allyl-TER was dissolved in glacial acetic acid and diluted with demineralized water to a final concentration of $5 \cdot 10^{-4}$ *M*. Tetrabutylammoniumbromide ($1 \cdot 10^{-3}$ *M*) was added to the sample as a mobility reference. The sample was injected hydrodynamically for 2 s ($3 \cdot 10^{3}$ Pa).

Experiments for the determination of the formation constants were performed at pH 4.9 and pH 2.2. BGEs at pH 4.9 consisted of 200 m*M* creatinine and 100 m*M* L(+)- or D(−)-mandelic acid or acetic acid. BGEs at pH 2.2 consisted of 5.00 m*M* aniline and 100 m*M* L(+)- or D(−)-mandelic acid or formic acid.

3. Results and discussion

Allyl-TER is a base with a pK_a-value of 7.1 [11]. At pH 4.9, the alkaloid is (almost) fully protonated, and will therefore migrate in the direction of the cathode. In order to calculate the formation constant, the effective mobility was determined as a function of the concentration of mandelate present in the BGE. Different concentrations of mandelic acid in the BGE were obtained by mixing BGEs containing mandelate with the BGEs containing acetate (pH 4.9) or formate (pH 2.2). In this way, unlike the method presented in [12], the ionic strength of the BGEs was independent of the mandelate concentration. This was very important in these experiments since very small differences in mobility had to be determined as accurate as possible, and it is well known that the ionic strength considerably influences the mobility. Since, especially at low pH values, the magnitude of the electroosmotic flow (EOF) can be difficult to determine accurately, tetrabutylammonium (TBA) was chosen as a mobility reference. This relatively slow cation is migrating closely to allyl-TER, and has no interaction with mandelate. The mobility of TBA was $14.2 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$ in the BGE containing creatinine (pH 4.9), and $14.5 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$ in the BGE containing aniline (pH 2.2). This difference in mobility is due to the higher ionic strength of the creatinine BGE. The mobility of allyl-TER is slightly lower than the mobility of TBA in these experiments and ranges in between 10 and $13 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$, depending on the composition of the BGE. The mobility of allyl-TER in absence of

mandelate (μ_0) is 13.0 in the BGE containing creatinine and 12.0 in the BGE containing aniline.

At pH 4.9, mandelic acid has a degree of dissociation $\alpha > 0.95$, since its pK_a value is 3.4. Therefore at this pH only ionoselective interaction [14] is assumed ($\alpha = 1$). The simple model of Wren [2] was used to determine the formation constants between allyl-TER and L(+)- and D(−)-mandelate. According to this model, the effective mobility of analytes, interacting with a CS (μ_a) is related to the formation constant (K_c), and the concentration of the CS ([C]):

$$\mu_a = \frac{\mu_0 + \mu_C K_C[C]}{1 + K_C[C]} \quad (1)$$

This can be rearranged to [15]:

$$\frac{\mu_0 - \mu_a}{[C]} = K_C(\mu_a - \mu_C) \quad (2)$$

For the sake of clarity, all symbols relevant to complex formation are listed and explained in Table 1. According to Eq. (2), a plot of $(\mu_0 - \mu_a)/[C]$ versus μ_a, should give a linear relationship with a slope equalling K_C and the μ_a-axis being intercepted at $\mu_a = \mu_C$. This is shown in Fig. 1a for L(+)- and D(−)-mandelic acid at pH 4.9. Formation constants and complex mobilities of L(+)- and D(−)-mandelate are presented in Table 2. These results show that the formation constants are low compared to complexes generally formed with cyclodextrins [2,4–6,15,16]. However, low formation constants have been reported previously, e.g. the formation constant between salbutamol and β-CD ($K_c = 9.6$) [17], and between benzylamine and either β-CD ($K_C = 9$) or heptakis (2,6-di-*O*-methyl-β-CD) ($K_C = 10$) [12]. Moreover, the order of magnitude of the formation constants, as determined in this study, is comparable to the results of our earlier study on the interaction between ergot alkaloids and organic acids [11]. The separation factor is very high: SF = 1.23. Standard errors are relatively high. This is explained by the fact that the decrease in mobility of the ergot alkaloid, due to interaction with mandelate, is very small. The mobility of the complex does not significantly differ from zero ($\mu_{C2} = 0$).

Next, the mobility of mandelate was determined at pH 2.2, in order to determine the magnitude of K_{C1}. At this pH, complex formation between mandelic acid and allyl-TER was not enantioselective because the mobility of allyl-TER did not differ significantly between BGEs consisting of (equal amounts of) L(+)- or D(−)-mandelic acid. The dissociation constant of mandelic acid is about 0.06, and as a rough estimation, we assume $\alpha = 0$. The results are presented in Table 2. In our earlier study [11] we assumed that only the dissociated acids interacted with the protonated ergot alkaloids. The current study proves that this assumption was not correct. However, it is true that only the dissociated acid interacts stereoselectively with the CS. This is a type II [4], or ionoselective [14] interaction according to the model of Rawjee et al. The formation constants

Table 1
Explanation of some symbols relevant for complex formation

Symbol	Explanation
μ_0 ($m^2 V^{-1} s^{-1}$)	Effective mobility of allyl-TER in absence of mandelic acid
μ_a ($m^2 V^{-1} s^{-1}$)	Effective mobility of allyl-TER in presence of mandelic acid
μ_{C1} and μ_{C2} ($m^2 V^{-1} s^{-1}$)	Effective mobility of the complex between allyl-TER and non-dissociated (μ_{C1}) and dissociated (μ_{C2}) mandelic acid
K_{C1} and K_{C2}	Formation constant for complexes between allyl-TER and non-dissociated (K_{C1}) and dissociated (K_{C2}) mandelic acid
[C] (*M*)	Concentration of mandelic acid in BGE
SF	Separation factor as defined by the ratio of the formation constants of both optical isomers

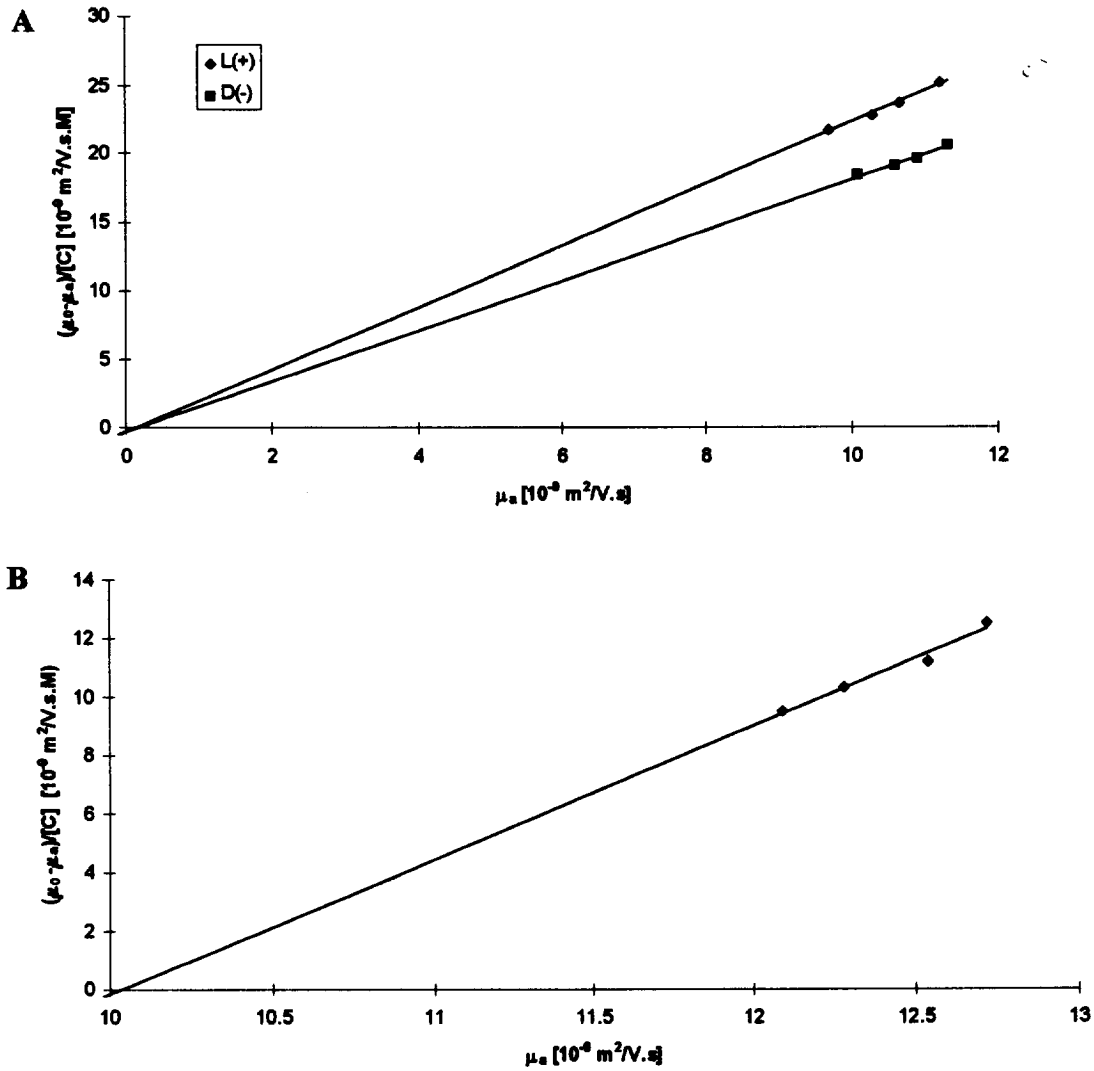

Fig. 1. Graphical determination of the formation constants of 1-allyl terguride with (a) both optical isomers of dissociated mandelic acid (BGE at pH 4.9) and (b) non-dissociated mandelic acid (BGE at pH 2.2). Experimental conditions are described in the text.

Table 2
Comparison between experimental data from this study (exp.) and [11]

	Average		Difference[a] [(+) vs. (−)]	
	Exp.	[11]	Exp.	[11]
K_{C1}	4.6 (0.5)	0	0%	0%
K_{C2}	2.05 (0.17)	6.00 (0.25)	21%	17%
μ_1 (10^{-9} m^2 V^{-1} s^{-1})	10 (0.3)	–	0%	–
μ_2 (10^{-9} m^2 V^{-1} s^{-1})	0.3 (1.9)	0	0%	0

Average formation constants with relative differences between both optical isomers, and mobility data for complexes of mandelic acid with allyl-TER, with standard deviations in parentheses.

[a] K_C[L(+)-mandelate] > K_C[D(−)-mandelate].

found in our earlier study are higher than the values obtained in the current study (Table 2). This could be explained by the fact that in this earlier study, experiments were performed at pH 4.2. The present study proves that at this pH, non-selective complex formation also occurs. Although the degree of dissociation at pH 4.2 is about 0.8, complex formation with the non-dissociated acid influences the mobility of the ergot alkaloid much more strongly than complex formation with the dissociated acid. This is clarified by Eq. (1), if the difference in complex mobility ($\mu_{C1} = +10$ versus $\mu_{C2} = 0$, with $\mu_0 = -22.3 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$ in the case where mandelate applied as analyte [11]) is taken into account. Therefore, formation constants (K_{C2}) presented in our earlier study were overestimated, since only interaction with the dissociated acids was taken into account. The difference in SF between current study (SF = 1.23) and our former study [11] (SF = 1.19) is not significant.

In an earlier study, Fanali et al. successfully separated ergot alkaloids using γ-cyclodextrins as chiral selector [18]. The present study proves that these alkaloids might also be separated using relatively inexpensive optically pure mandelic acid as counter-ion. Fig. 2 shows the optimized separation of a mixture of (−)-TER: (+)-TER = 1:1.5. This separation was achieved by applying a BGE containing 100 m*M* L(+)-mandelic acid at pH 4.5. At this pH, the degree of dissociation of mandelic acid is $\alpha > 0.9$. (+)-TER is the slowest migrating alkaloid, and therefore we can conclude that (+)-TER has the strongest interaction with L(+)-mandelate, confirming our earlier results. Reversal of the migration order was easily accomplished by using D(−)-mandelate instead of L(+)-mandelate as counter-ion.

4. Conclusions

The presented method proved to be suitable for an accurate determination of even very low formation constants. The results presented confirmed our earlier observations that only the dissociated acids interact stereoselectively with the ergot alkaloid. The interaction between the alkaloid and the non-dissociated acid is not stereoselective, however, formation constants (K_{C1}) are higher than those of the stereoselective interactions (K_{C2}) with the dissociated acids.

Using an analogous experimental set-up it was possible to separate the optical isomers of terguride applying optically pure mandelic acid as chiral selector. This method is cheaper and gives better separation than an earlier study applying γ-cyclodextrin. Moreover, it is very easy to reverse the migration order of the terguride optical isomers by using D(−)-mandelate instead of L(+)-mandelate as counter ion.

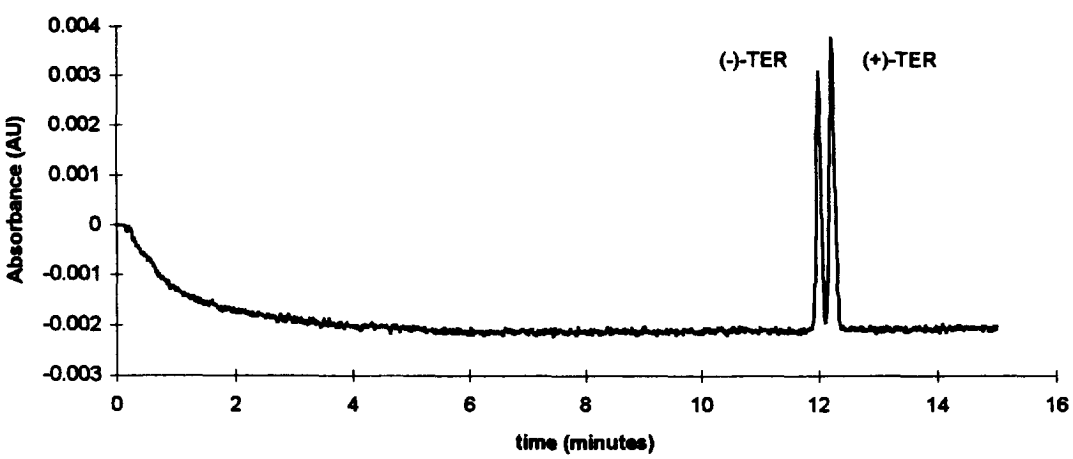

Fig. 2. Chiral separation of a mixture of terguride (TER) enantiomers; (−)-TER: (+)-TER = 1:1.5. BGE: 200 m*M* ε-aminocapronic acid, 100 m*M* L(+)-mandelic acid (pH 4.5). Capillary 400–470 mm, I.D. 50 μm, polyacrylamide coated. Detection: 280 nm. Separation voltage: 20 kV. Temperature: 17°C.

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