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Separation of diastereomeric anthrone-C-glucosyls of aloes by micellar electrokinetic capillary chromatography

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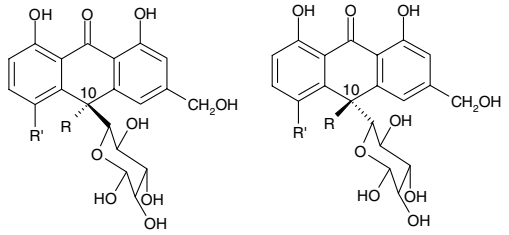
The anthrone-C-glucosyls aloin A and B, 5-hydroxyaloin A, 10-hydroxyaloin A and B were separated by micellar electrokinetic capillary chromatography (MECC) with sodium dodecyl sulfate in borate buffer pH 9 within less than 7 min. The method has been successfully transferred to the analysis of the two European Pharmacopoeia drugs Cape aloes and Curaçao aloes. A comparison of the peak areas received by HPLC and MECC indicated the transferability of the measured contents.

The term "Aloe" stands for a taxonomic genus of the Asphodelaceae (Smith et al. 1991). The European Pharmacopoeia (Ph.Eur.) lists two drugs, Cape aloes (the dried sap of the leaves from in particular the species *Aloe ferox* and its hybrids) and Curaçao aloes (the dried sap of the leaves from the species *Aloe barbadensis*), containing at least 18.0 resp. 28.0 per cent hydroxyanthracene derivatives calculated as aloin. The content of aloin is still specified by measuring the absorbance after several complicated steps of extraction, although various simpler methods by HPLC and capillary electrophoresis (CE) have been developed during the last years (Suzuki et al. 1986; Rauwald and Beil 1993a; Kuzuya et al. 2001). The main components of the two pharmaceutical drugs are the diastereomers aloin A (10*S*, 1′*S*) and B (10*R*, 1′*S*) (Rauwald and Lohse 1989). Additional native characteristic derivatives (e.g. 5-hydroxyaloin A – naturally occurring in only one configuration (Rauwald and Beil 1993a) – in Cape aloes, 7-hydroxyaloin A and B in Curaçao aloes (Rauwald and Voetig 1982) and aloe-emodin in both) are found in minor concentrations.

In the analysis of drugs and plants the CE is of increasing interest (Wätzig and Dette 1994; Nishi 1999) because of its many advantages compared to HPLC on certain issues. Small amounts of buffer and sample, a high separation efficiency and a short time of analysis are only a few of them. One method of high potential and easy handling is the micellar electrokinetic capillary chromatography (MECC). For some hydroxyanthracene derivatives CE-methods have been established by several groups (Sheu and Chen 1995; Sheu and Lu 1995; Weng and Sheu 2000). There are basic approaches for the separation of the anthrone-C-glucosyls aloin A and B (Kuzuya et al. 2001), but to our knowledge neither 5-hydroxyaloin A nor 10-hydroxyaloin A and B, the main *in vitro* oxidation products of the aloins (Rauwald and Lohse 1992), have been determined by CE yet. In the present work, we searched for a rapid and easy separation method for the analysis of aloe drugs.



aloin A: R = R' = H
5-OH-aloin A: R = H, R' = OH
10-OH-aloin A: R = OH, R' = H

aloin B: R = R' = H
10-OH-aloin B: R = OH, R' = H

With this new method, an optimal separation was obtained by using a 30 mM borate buffer with 20 mM sodium dodecyl sulfate (SDS), pH 9. Variant SDS concentrations led to blurred peaks and worse separation results, in particular, lower concentrations resulted in current leakage and baseline drifting. Without the addition of SDS, a separation of aloin A and B could not be obtained. An increase of pH up to 10.5 declined the separation. A temperature of 15 °C was associated with baseline and current stability. The samples were analyzed as shown in the Fig. by typical electropherograms, and their migration times were compared. The electroosmotic flow (EOF) was detected by using methanol as marker. 4-Hydroxybenzaldehyde was used as an internal standard, absorbing at 360 nm. The relative migration times of aloin A (1.355 ± 0.017) and B (1.325 ± 0.017) in comparison with 4-hydroxybenzaldehyde (1.000) were assayed ($n = 9$). The 10-hydroxyaloin sample (Fig. C) contained high quantities of apolar substances like aloe-emodin and chrysophanol, apart from the 10-hydroxyaloin A and B plus aloin A and B sample, as it had not been purified after oxidation. The samples of Cape aloes and Curaçao aloes were compared with the 5-hydroxyaloin A sample. 5-Hydroxyaloin A was found in Cape aloes, but not in Curaçao aloes. Its migration time lies between the 10-hydroxyaloins A and B, which have not been found in those samples. The ratio of aloin B to A was measured by HPLC (0.749) and compared to that measured by MECC (0.752).

Aloin A and B were separated by MECC with almost baseline separation. The generated method allows a simple determination of anthrone-C-glucosyls in samples, which is with less than 7 min a lot faster than the established HPLC methods and to our knowledge faster than the other MECC methods suggested so far. The higher the polarity of the substance the lesser it is retarded by interaction with the negatively charged micelles. Therefore the hydroxyaloins show shorter migration times than the aloins. As diastereomers aloin A and B differ only at C-10 in their stereochemistry, nevertheless aloin B has a little higher polarity than aloin A, making the separation with MECC possible. 4-Hydroxybenzaldehyde has been found to be a convenient internal standard for anthrone-C-glucosyl se-

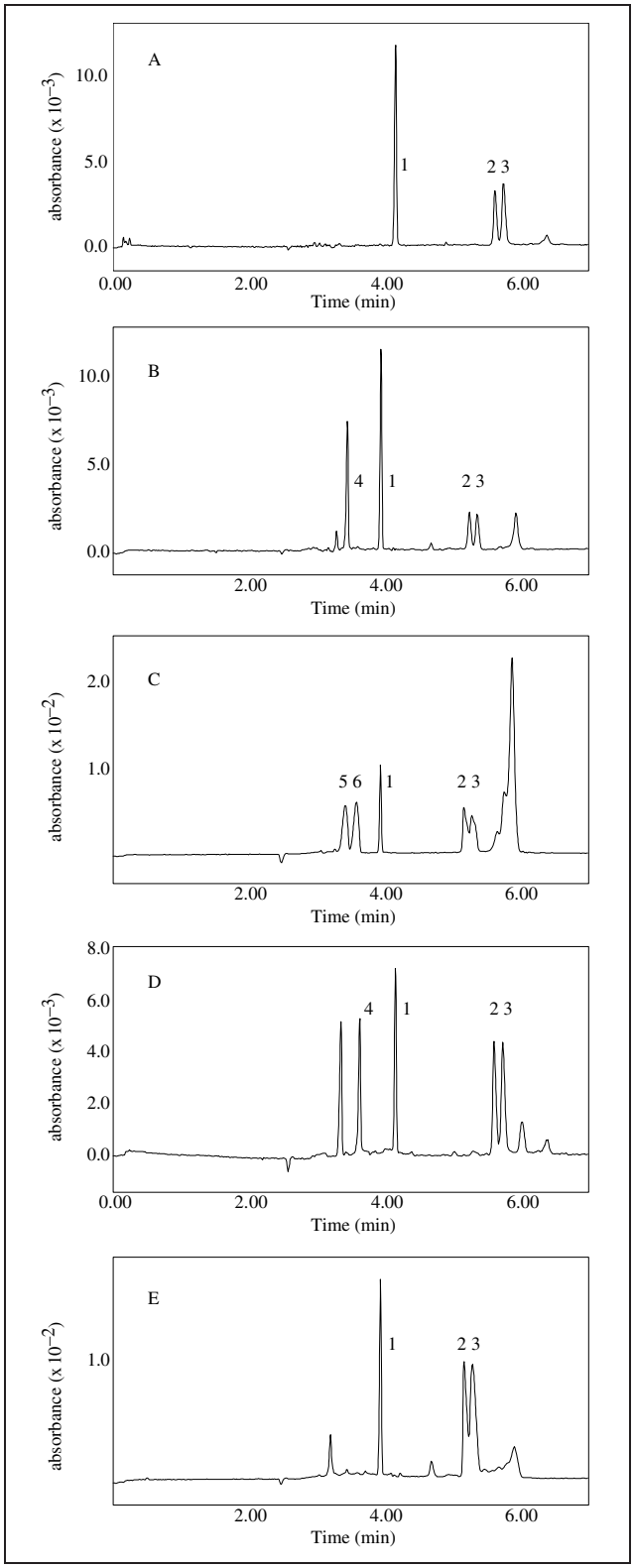


Fig.: MECC separation of (A) aloin, (B) 5-hydroxyaloin, (C) 10-hydroxyaloin, (D) Cape aloes, (E) Curaçao aloes
(Peak 1: 4-hydroxybenzaldehyde, 2,3: aloin B and A, 4: 5-hydroxyaloin A, 5,6: 10-hydroxyaloin B and A);
MECC conditions: capillary: uncoated fused silica capillary (75 μm × 40 cm), temperature: 15 °C, UV detection set at 360 nm, applied voltage: 15 kV, buffer: SDS 20 mM in borate 30 mM, pH 9.0

paration by MECC, as it absorbs with a characteristic spectrum and is detectable at 360 nm. Its migration time lies between the examined substances without overlapping and has reproducible migration time ratios. Because there is no 5-hydroxyaloin in Curaçao aloes it is possible to differentiate the Ph.Eur. drugs by CE. Additional benefit is the possibility of controlling the progress of 10-hydroxyaloin synthesis from the aloins A and B. The comparison of areas detected by HPLC and CE showed the option to determine the content of aloe drugs by using this method.

Experimental

1. Chemicals

The following HPLC grade chemicals were used: sodium hydroxide solution 0.1 M (Grüssing GmbH, Filsum, Germany); sodium hydroxide (Lachema, Brno, Czech Republic); boric acid, 4-hydroxybenzaldehyde > 98% for synthesis (Merck, Darmstadt, Germany); methanol LC-MS-grade, sodium dodecyl sulfate (SDS) ultra pure ≥ 99% (Roth, Karlsruhe, Germany). Water was distilled after deionisation. All samples, except for 'Aloinum recrystallized' Ph.Brit. 1914 (E. Merck, Darmstadt, Germany), were available in our laboratory: 5-hydroxyaloin A, Cape aloes from Mosselbay and Curaçao aloes Ph.Eur.III quality; 10-hydroxyaloin was synthesized from aloin according to Rauwald and Lohse (1992).

2. Equipment

CE separations were carried out using a P/ACE™ System MDQ (Beckman Instruments Inc., Fullerton, USA) with a multichannel UV-Vis diode array detector (Beckman Instruments Inc., Fullerton, USA), software Beckman P/ACE™ System MDQ version 2.3, uncoated fused-silica capillary 75 μm i.d., 375 μm o.d. eCap™ (Beckman Instruments Inc., Fullerton, USA) with an effective length of 30 cm to the detection window (40 cm total length), 100 × 800 μm aperture size. The HPLC analyses were performed on an EC 250/4 Nucleosil™ 100-5 c18 column (Macherey-Nagel, Düren, Germany) with two Dynamax sd-300 pumps (Rainin Instruments, Woburn, USA). A Dynamax UV1 detector (Rainin Instruments, Woburn, USA) was used, software: Rainin Dynamax (Rainin Instruments, Woburn, USA).

3. Buffer preparation

For MECC, a 30 mM borate buffer pH 9.0 (made by solving boric acid in water and titrating it with sodium hydroxide solution 0.1 mM) with an additive of 20 mM SDS as surfactant was filtered through a 0.45 μm cellulose nitrate filter (Sartorius, Göttingen, Germany).

4. Sample preparation

For the analysis of aloin A and B 1.0 ml of methanol was added to 0.5 mg aloin and then diluted 3 + 17 with water. 5-Hydroxyaloin (0.45 mg) was solved in 1.0 ml methanol and then diluted 1 + 3. The 10-hydroxyaloin sample was prepared by diluting a 10-hydroxyaloin A and B containing solution (12.5 mg/ml) 1 + 19. Cape aloes and Curaçao aloes were extracted with methanol-water (1 + 1) each 50.0 mg in 16.7 ml for 15 min, then filtered through a 0.22 μm membrane filter and diluted 1 + 5 resp. 3 + 17. A second time, every sample was prepared with 4-hydroxybenzaldehyde (0.05 mg/ml) as internal standard. The samples were proved by TLC (Rauwald and Beil 1993b); the ratio of aloin A and B in the aloin sample was measured by HPLC (Rauwald and Beil 1993a).

5. Capillary electrophoresis

Before initiating a series of measurements the capillary was activated by rinsing 0.1 M NaOH, then water and buffer. Every run was started with buffer rinsing (2 min, 137.9 kPa); sample injection was performed with 3.4 kPa over 3 s, then separation lasted 7 min (15 kV, 0.17 s ramp time, average current 25 mA) at 15 °C. The substances were detected at 360 nm and spectra were recorded per PDA-detector to identify the peaks independently from their migration time.

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New pomolic acid triterpene glycosides from *Zygophyllum eichwaldii*

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Two new bisdesmosidic triterpenoid saponins, 3-*O*-[α-L-2-*O*-sulphonylarabinopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygoeichwaloside H) and 3-*O*-[β-D-2-*O*-sulphonylglucopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygoeichwaloside K) were isolated from the roots of *Zygophyllum eichwaldii*. The structures were established primarily on the basis of NMR spectroscopy and chemical transformations.

As a part of our continuing phytochemical research on plants of the genus *Zygophyllum* which are used in the traditional medicine of Asian countries (Sasmakov et al. 2001; 2003), this paper deals with the isolation and structural elucidation of two new triterpenoid saponins from *Zygophyllum eichwaldii*.

Separation of triterpene-containing fractions of the methanolic extract of the roots of *Zygophyllum eichwaldii* on a silica gel column (gradient chloroform, methanol and water) led to the isolation of two triterpenes (**1** and **2**). Saponin **1** was obtained as an amorphous white powder. IR absorptions at 3410, 1734 and 1648 cm^{-1} indicated the presence of hydroxyl (OH), ester carbonyl (C=O), and double bond (C=C) functionalities. The olefinic resonances of the aglycone at δ 128.05 and 138.97, corresponding to quaternary and methine carbons suggested the urs-12-ene skeleton with a hydroxyl group at C-19 (Inada et al. 1987; Ouyang et al. 1997). The ^{13}C NMR spectral data of **1** were consistent with pomolic acid as the aglycone (Sasmakov et al. 2001). 41 different signals in the ^{13}C NMR spectrum supported that **1** has a bisdesmosidic structure (Table). This is confirmed by availability of hydrogen anomeric atoms at δ 5.10 and δ 6.32 in the ^{1}H NMR spectra. Acidic hydrolysis of the glycoside yielded pomolic acid as aglycone and arabinopyranose and glucopyranose as sugar parts. The ^{13}C NMR spectrum of **1** contained signals at δ 89.41 attributable to C-3 and showing that the hydroxyl group at this carbon is glycosylated. The signals of C-1′ carbon atom arabinose at δ 103.60 and H-1′ proton at δ 5.10 showed that the arabinose is located at C-3 of the aglycone. The downfield shifts of the H-2′ and C-2′ signals of arabinose compared with those of Ziyu-glycoside I (**3**) (Table) (Sasmakov et al. 2001; Yosioka et al. 1970) indicated that the sulphate group was in position C-2′ of the arabinose. The presence of the $-SO_3H$ group was con-