Journal of Chromatography, 639 (1993) 359–362
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 038

Short Communication

High-performance liquid chromatographic separation and determination of diastereomeric anthrone-C-glucosyls in Cape aloes

Hans W. Rauwald* and Anette Beil

Institute of Pharmaceutical Biology, Johann Wolfgang Goethe-University, Georg-Voigt-Strasse 16, W-6000 Frankfurt M. 11 (Germany)

(First received November 24th, 1992; revised manuscript received March 5th, 1993)

ABSTRACT

Isocratic high-performance liquid chromatography using reversed-phase packing (C_{18}) and the solvent system methanol–water (1:1) was successful in separating and determinating the anthrone-C-glucosyls in Cape aloes, allowing a rapid and high resolution of each diastereomer for the first time. By applying this method to fifteen commercial Cape and East African aloes of different origin, all Cape aloes samples showed the same anthrone-C-glucosyl pattern comprising 5-hydroxyaloin A, aloins A/B and aloinosides A/B. For differentiating non-official East African aloes from Cape aloes, 5-hydroxyaloin A proved to be the most specific marker of the official drugs in high-performance liquid and also thin-layer chromatography, replacing the aloinosides. Previous assumptions of chemical races of *Aloe ferox* MILL. based on the aloinosides have to be revised.

INTRODUCTION

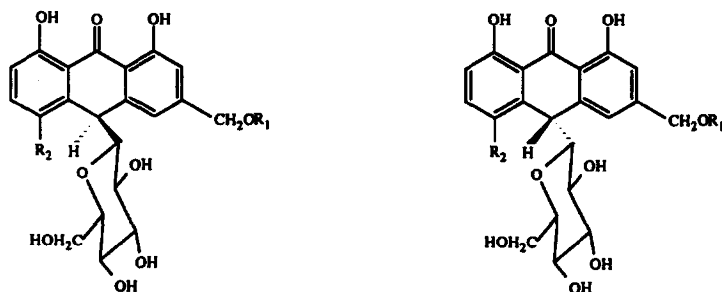
Cape aloes (derived from *Aloe ferox* MILL. and hybrids, *Asphodelaceae*) is a remedy used worldwide. It is listed in numerous pharmacopoeias because of its purgative activity. The active principle comprises five anthrone-C-glucosyls [1] (Fig. 1): the diastereomeric aloins A/B and aloinosides A/B and the so-called "periodate-positive substance" [2], the structure of which we have recently determined to be 5-hydroxyaloin A [3].

As yet no study has simultaneously determined all five anthrone-C-glucosyls by high-performance liquid chromatography (HPLC). Although in 1979 a reversed-phase HPLC separation with 45% aqueous methanol was described for the aloins [4], all subsequent publications on the HPLC determination of anthrone-C-glucosyls from *Aloe* dealt with the analysis of aloins [5–7] or homonataloins [8].

In our study we describe a simple and rapid isocratic reversed-phase HPLC method for the baseline separation, identification and assay of aloins A/B, aloinosides A/B and 5-hydroxyaloin A in aloes. The method was applied to fifteen commercial Cape and East African aloes of different origin. It ensured an exact determination of the characteristic pattern of diastereomeric anthrone-C-glucosyls in Cape aloes.

* Corresponding author.

0021-9673/93/$06.00 © 1993 Elsevier Science Publishers B.V. All rights reserved



	R_1	R_2	Configuration (10, 1'[17])
Aloin A	H	H	S, S
Aloin B	H	H	R, S
Aloinoside A	α-L-rhamnosyl	H	S, S
Aloinoside B	α-L-rhamnosyl	H	R, S
5-Hydroxyaloin A	H	OH	R, S

Fig. 1. Diastereomeric anthrone-C-glucosyls from Cape aloes.

EXPERIMENTAL

Solvents

All solvents were of technical quality and were purified by distillation.

High-performance liquid chromatography

Apparatus. The equipment consisted of a Waters 600 solvent-delivery system (Waters, Milford, MA, USA) and a U6K injector. A Waters 990 photodiode-array detector coupled with a NEC APC IV personal computer (NEC Information Systems, Boxborough, MA, USA) was used for recording UV–VIS spectra (200–500 nm) of the separated compounds and standard compounds and for controlling retention time at 360 nm.

Conditions. Separations were performed on an ET 250/8/4 Nucleosil 7 C_{18} column (Macherey, Nagel, Düren, Germany) at room temperature. The mobile phase consisted of methanol–water (1:1); the flow-rate was 1 ml/min. Each sample was chromatographed three times. The injection volume was 20 μl.

Sample preparation. A 300-mg aliquot of the powdered drug was dissolved in exactly 100 ml of methanol–water (1:1) by automatic shaking for 15 min. An aliquot of these solutions was filtered through a cellulose acetate filter (0.45 μm; Lida, Kenosha, WI, USA).

Standard samples. 5-Hydroxyaloin A was isolated according to a previously reported procedure [3]. The aloinosides A and B could be obtained from Cape aloes by preparative thin-layer chromatography (TLC) in the solvent system ethylacetate–methanol–water (100:17:13); for TLC identification of aloinosides, see refs. 9–11. Preparative separation of the aloins A and B was achieved by droplet countercurrent chromatography [12].

Precision. A solution of 100 mg of aloins A/B in 100 ml of 50% aqueous methanol was treated as described in the Sample preparation section and was chromatographed five times. The results obtained were also compared with the HPLC separation of an unfiltered solution of 100 mg of aloins A/B in 100 ml of methanol.

Thin-layer chromatography

TLC separations on silica gel F_{254} plates (Macherey, Nagel) were carried out according to the European Pharmacopoeia (Ph.Eur.).

By using chloroform–methanol–water (7:13:8; lower phase) as the solvent system, all five diastereomers can be detected separately [13]. 5-Hydroxyaloin A was detected by spraying with 5% aqueous sodium metaperiodate (Merck, Darmstadt, Germany).

RESULTS AND DISCUSSION

In order to obtain a rapid and high resolution of 5-hydroxyaloin A and aloinosides A/B the HPLC parameters described for the aloins [4] had to be modified with respect to particle size of the stationary phase, solvent system and flow-rate. This led to an isocratic baseline separation of all five diastereomers for the first time: within about 20 min each diastereomer could be determined in a single HPLC run (Fig. 2). Thus, the technique not only replaces the aloes identification and assay of Ph.Eur., but can also be applied to pharmacokinetic and toxicological studies, which have been required for aloes by the German health administration since May 1992 [14].

In contrast to the assay of Ph.Eur., the pulverized drugs were dissolved directly in methanol–water (1:1), filtered and chromatographed on reversed-phase packing (C_{18}) with methanol–water (1:1). For the aloins A/B the average recovery was 97.2% with a relative standard deviation of 0.6% ($n = 5$). In comparison to our sample preparation, the complicated but conventional Ph.Eur. solving method showed no difference. A specific peak identification was achieved by means of photodiode-array detection (comparison of spectra and control of retention time at 360 nm). In routine analysis, UV detection at 360 nm is sufficient.

In a screening of fifteen commercial drugs, this HPLC technique enabled us to monitor the characteristic pattern of anthrone-C-glucosyls in each sample. Table I shows the content of each separated anthrone-C-glucosyl. A listing of the drugs according to their origin agrees with their arrangement according to their qualitative and for the most part quantitative anthrone-C-glucosyl pattern: Cape aloes samples from the Mosselbay region (South Africa) show high contents of all five anthrone-C-glucosyls. In this case all five compounds can also be well detected by means of TLC.

5-Hydroxyaloin A is weakly detectable by means of TLC (applied amounts according to Ph.Eur.) in Cape aloes samples 8–10 from Port Elizabeth (South Africa). The Ph.Eur. TLC markers aloinoside A and B cannot be determined at all by TLC. This finding led to the commonly accepted assumption of aloinoside-free chemivars of *Aloe ferox* [9,15]. However, the qualitative and quantitative determination by HPLC shows positive proof of aloinosides A and B in Port Elizabeth samples for the first time. A differentiation of *Aloe ferox* in several chemivars could thus only be discussed with regard to the quantitative pattern of the aloinosides A and B.

East African aloes (samples 12–15) are still available commercially. It is shown for the first time that all drugs of East African origin lack 5-hydroxyaloin A (Table I) and that some of them contain more aloinosides than aloins.

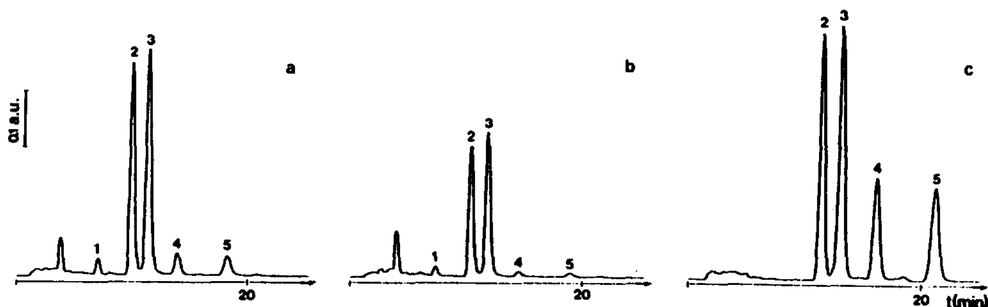


Fig. 2. Reversed-phase high-performance liquid chromatograms at 360 nm of (a) Cape aloes from Mosselbay, (b) Cape aloes from Port Elizabeth and (c) Kenyan aloes. Conditions were as described in the Experimental section. Peaks: 1 = 5-hydroxyaloin A; 2 = aloin B; 3 = aloin A; 4 = aloinoside B; 5 = aloinoside A.

TABLE I

QUANTITATIVE DETERMINATION (%) OF 5-HYDROXYALOIN A, ALOINS A/B AND ALOINOSIDES A/B IN ALOES OF DIFFERENT ORIGIN

Sample	Origin	5-Hydroxyaloin A	Aloin A	Aloin B	Aloinoside A	Aloinoside B	Sum
1–4	Mosselbay	1.7–2.9	13.0–13.9	10.9–12.0	3.1–3.9	2.8–3.6	31.7–34.9
5–7	Quality "Ph.Eur."	1.1–2.3	12.7–13.1	10.8–11.3	2.6–3.4	2.4–3.1	31.3–31.8
8–10	Port Elizabeth	0.3–0.8	6.5– 8.5	5.2– 7.1	0.3–0.9	0.4–0.8	13.3–17.8
11	Port Elizabeth	3.3	12.5	10.8	4.0	3.5	34.1
12	Kenya	–	16.3	14.0	19.6	16.9	66.8
13	East Africa	–	7.3	5.8	13.6	11.7	38.4
14	East Africa	–	14.8	11.9	2.9	2.5	32.1
15	East Africa	–	12.7	10.6	6.2	5.1	34.6

Whereas for samples 13–15 the specific origin was not declared, sample 12 was stated to be "Kenyan Cape aloes". This classification must be called in question because of the lack of 5-hydroxyaloin A, especially since *Aloe rabaiensis* RENDLE is reported to be the origin of Kenyan aloes [16]. The Ph.Eur. TLC method does not permit a differentiation between East and South African aloes, because it only monitors the aloins and aloinosides. Therefore 5-hydroxyaloin A is proposed as most specific TLC marker, replacing the aloinosides. Furthermore, our HPLC results confirm that 5-hydroxyaloin is until now the only C-glucosyl-anthrone that has not been found as a diastereomeric pair genuinely in aloes: It only occurs in the more stable A-configuration (10*R*, 1′*S* [17]).

In summary, the characteristic anthranoid pattern of Cape aloes —including Cape aloes from Port Elizabeth— comprises all five anthrone-C-glucosyls. As already pointed out, these five glucosyls cannot be detected in each case, following Ph.Eur. TLC conditions. However, the more sensitive and rapid HPLC method allows a definite determination of each anthrone-C-glucosyl in a single working step.

ACKNOWLEDGEMENTS

We are grateful to Caesar & Loretz GmbH, Hilden (Germany), Alfred Galke GmbH, Gittelde (Germany), Heinrich Klenk GmbH, Schweinfurt (Germany), Paul Müggenburg GmbH & Co., Alveslohe (Germany), and Petereit & Co. GmbH, Elmshorn (Germany) for providing the drug material.

REFERENCES

1 R.K. Mapp and T.J. McCarthy, *Planta Med.*, 18 (1970) 361.
2 H. Böhme and L. Kreutzig, *Dtsch. Apoth. Ztg.*, 103 (1963) 505.
3 H.W. Rauwald, *Pharm. Weekbl. Sci. Ed.*, 9 (1987) 215.
4 M. Grün and G. Franz, *Pharmazie*, 34 (1979) 669.
5 H. Auterhoff, E. Graf, G. Eurisch and M. Alexa, *Arch. Pharm. (Weinheim)*, 313 (1980) 113.
6 E. Graf and M. Alexa, *Arch. Pharm. (Weinheim)*, 313 (1980) 285.
7 Q.J. Groom and T. Reynolds, *Planta Med.*, 53 (1987) 345.
8 J. Beaumont, T. Reynolds and J.G. Vaughan, *Planta Med.*, 50 (1984) 505.
9 L. Hörhammer, H. Wagner and G. Bittner, *Arzneimittel-Forsch.*, 13 (1963) 537.
10 L. Hörhammer, H. Wagner and G. Bittner, *Z. Naturforschg.*, 19b (1964) 222.
11 A. Beil and H.W. Rauwald, in R. Hänsel, K. Keller, H. Rimpler and G. Schneider (Editors), *Hagers Handbuch der Pharmazeutischen Praxis*, Vol. 4, Springer, Berlin, 1992, p. 216.
12 H.W. Rauwald, K. Lohse and J.W. Bats, *Angew. Chem.*, 101 (1989) 1539.
13 H.W. Rauwald, *Arch. Pharm. (Weinheim)*, 315 (1982) 769.
14 Anonymous, *Dtsch. Apoth. Ztg.*, 132 (1992) 1164.
15 R. Hegnauer, *Chemotaxonomie der Pflanzen*, Vol. 7, Birkhäuser, Basle, 1986, p. 705.
16 J. Conner, A. Gray, T. Reynolds and P. Waterman, *Pharm. Weekbl. Sci. Ed.*, 9 (1987) 216.
17 H.W. Rauwald, *Pharm. Ztg. Wiss.*, 3/135 (1990) 169.