

# 5-Hydroxyaloin A in the Genus *Aloe*

## Thin Layer Chromatographic Screening and High Performance Liquid Chromatographic Determination

Hans W. Rauwald and Anette Beil

Institut für Pharmazeutische Biologie, Johann Wolfgang Goethe-Universität,  
Georg-Voigt-Straße 16, D-W-6000 Frankfurt am Main 11, Bundesrepublik Deutschland

Z. Naturforsch. **48c**, 1–4 (1993); received December 12, 1992

*Aloe*, Asphodelaceae, 5-Hydroxyaloin A, Quinones, Chemotaxonomy

By a comparative thin layer chromatographic screening of the methanol-soluble leaf exudates from more than 400 *Aloe* plants (183 species), 5-hydroxyaloin A was identified in 20 species. Whilst 13 of the 20 species revealed interindividual variations concerning to the occurrence of 5-hydroxyaloin A, this anthrone-C-glucosyl was unambiguously detected in each individual of 6 *Aloe* species. In the leaf exudates from *A. marlothii* Berger 5-hydroxyaloin A was only traceable in the aloin-containing chemivars. The complete anthrone-C-glucosyl pattern of these 7 clearly characterized species has been determined additionally by qualitative and quantitative high performance liquid chromatography: The results obtained demonstrate that 5-hydroxyaloin only occurs in the more stable A-configuration (10*R*, 1'*S*), thus being till now the only anthrone-C-glycosyl which has not been found as diastereomeric pair genuinely in plants. As well, 5-hydroxyaloin A characterizes a quantitatively significant hydroxylating pathway in biosynthesis of anthranoids. It is discussed as a chemotaxonomic marker of the genus *Aloe*, especially of the sections *Pachydendron* and *Eualoe*.

### Introduction

5-Hydroxyaloin A (**1**; Fig. 1) [1] – the so-called “periodate-positive substance” [2] – is a characteristic anthrone-C-glucosyl from Cape aloes [3]. The worldwide used drug is listed in numerous pharmacopeias because of its purgative activity. The active principle comprises diastereomeric anthrone-C-glucosyls including **1** [4]. Referring to our

observations on the phytochemistry of commercial Cape aloes drugs [1, 3], so far **1** is the only anthrone-C-glucosyl which has not been found genuinely as diastereomeric pair, but only in the more stable A-configuration (10*R*, 1'*S*) [5].

The genus *Aloe* (Asphodelaceae) consists of more than 360 species [6]. To achieve a chemotaxonomic characterization, anthrone-C-glucosyls are considered to be the most specific secondary products in TLC screenings of *Aloe* [7–9]. The occurrence of **1** in the genus has not been studied previously. We now present a comparative TLC screening of the MeOH-soluble leaf exudates from more than 400 *Aloe* plants representing 183 *Aloe* species. The extracts were analyzed for **1** and related anthrone-C-glucosyls of the aloin- and 7-hydroxyaloin type. Based on our TLC results, the quantitative significance of **1** in anthranoid metabolism of 7 *Aloe* species was determined by reversed phase HPLC.

### Materials and Methods

#### TLC screening

Leaf material was obtained from 11 German botanical gardens; an exact description can be found in [9]. Most *Aloe* species were represented by leaf material from several individuals of different bo-

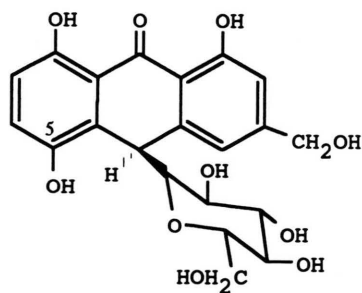


Fig. 1. 5-Hydroxyaloin A.

**Abbreviations:** TLC, thin layer chromatography; HPLC, high performance liquid chromatography.

Reprint requests to Prof. Dr. H. W. Rauwald.

Verlag der Zeitschrift für Naturforschung,  
D-W-7400 Tübingen  
0939–5075/93/0100–0001 \$ 01.30/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

tanical gardens. Extraction and sample preparation were carried out as described by [8]. TLC plates: silica gel UV<sub>254</sub> (Macherey-Nagel & Co., Düren, F.R.G.); solvent systems: CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (7:13:8, lower phase; permits separation of diastereomeric anthrone-C-glucosyls [10]) and CHCl<sub>3</sub>–MeOH (50:10/15/20; repeated TLC with increasing polarity for specific identification of **1**). Detection: **1**, aloins A/B, aloinosides A/B and the anthrone-C-glucosyls of 7-hydroxyaloin type (homonataloins A/B, 7-hydroxyaloin A/B, 7-hydroxyaloin-6'-monoacetates and -4',6'-diacetates A/B, 8-O-methyl-7-hydroxyaloin A/B and their 6'-cinnamoyl esters) were identified with authentic reference substances which are available in our laboratory. Under UV<sub>366</sub> all hydroxylated anthrone-C-glucosyls appear as dark-violet spots. After spraying with 5% aqueous sodium metaperiodate these compounds stain violet in daylight. **1** is the only hydroxylated anthrone-C-glucosyl that stains violet-brown with sodium metaperiodate; the colour disappears in a few minutes [2]. For retention-to-front values compare [6].

### HPLC

Fresh leaves from *A. broomi*, *A. ferox*, *A. khamiensis* and *A. microstigma* were collected in the Palmengarten Frankfurt/M. (F.R.G.) in April 1990. Leaf samples of *A. aculeata*, *A. longistyla* and *A. marlothii* were collected in the Botanical Garden of Heidelberg (F.R.G.) in May 1990. The leaves were cut from a middle position relative to

the apex and were deep-frozen as soon as possible. Sample preparation: Freeze-dried and powdered leaf material was extracted three times for 30 min with MeOH at room temperature by automatic shaking. Filtrates were evaporated to dryness under vacuum at 20 °C. For HPLC, residues were dissolved in an aliquot of 50% aqueous MeOH. These solutions were filtered through cellulose acetate filter (0.45 µm). Detailed HPLC conditions are described in [3].

### Results and Discussion

In the course of our TLC screening program **1** was clearly identified in the MeOH-soluble leaf exudates from different individuals of the 7 *Aloe* species specified in Table I. In the following 13 *Aloe* species **1** was not reproducibly detectable due to interindividual variations: section Pachydendron: *A. africana* Mill., *A. candelabrum* Berger, *A. excelsa* Berger, *A. petricola* Pole Evans, and *A. reitzii* Reynolds; section Eualoe/subsection Humiles: *A. brevifolia* Mill. and *A. pretoriensis* Pole Evans; Eualoe/Grandes: *A. harlana* Reynolds; Eualoe/Prolongatae: *A. cameronii* Helms, *A. distans* Haw., and *A. pendens* Forsk.; section Aloidendron: *A. bainesii* Th. Dyer; not classified: *A. pictifolia* Hardy. Reasons for these variations were not determined. They may be based on unrecognized hybridization or different growing conditions. It is also known that certain *Aloe* species form populations with distinct anthranoid patterns [8, 11].

Table I. Content (in %, referring to dry weight) of 5-hydroxyaloin A, aloins A/B and aloinosides A/B in methanolic leaf extracts from 7 *Aloe* species.

<i>Aloe</i> species (section/subsection)	5-Hydroxy- aloin A	Aloin A	Aloin B	Aloinoside A	Aloinoside B
<i>A. aculeata</i> Pole Evans (Pachydendron)	0.2	0.2	0.2	0.2	0.2
<i>A. ferox</i> Mill. (Pachydendron)	0.4	0.7	0.6	0.5	0.5
<i>A. marlothii</i> Berger (Pachydendron)	0.3	0.1	0.1	–	–
<i>A. broomii</i> Schoenl. (Eualoe/Parvae)	4.1	2.3	2.0	–	–
<i>A. longistyla</i> Bak. (Eualoe/Parvae)	0.1	–	–	–	–
<i>A. khamiensis</i> Pillans (Eualoe/Magnae)	6.6	–	–	–	–
<i>A. microstigma</i> Salm Dyck (Eualoe/Magnae)	1.4	–	–	–	–

Our TLC screening shows that **1** is not limited only to Cape aloes or its origin *A. ferox*, but also occurs in some further *Aloe* species, particularly in members of the sections *Pachydendron* and *Eualoe* (classification according to Jacobsen [12] on the basis of Reynolds [13]). In contrast to the widespread anthrone-C-glucosyls aloins A/B and homonataloins A/B [9] **1** may be regarded as a chemotaxonomic marker. Further investigations are necessary to confirm the specific occurrence of **1** in the sections mentioned. Concerning the metabolism of anthrone-C-glucosyls in *Aloe*, the TLC screening makes obvious that the 5-hydroxylating pathway is interindividually separated from the 7-hydroxylating step: **1** and the glucosyls of 7-hydroxyaloin type never occur together in one plant. **1** is either the only detectable anthrone-C-glucosyl in the leaf exudate or coincides with the aloins A/B and aloinosides A/B. A vicarious occurrence of aloins A/B and homonataloins A/B has been reported previously for *A. marlothii* [8]. In addition, **1** is only traceable in the aloin-containing chemivars of *A. marlothii*. This aspect supports the separation of 5- and 7-hydroxylating step in biosynthesis of anthrone-C-glucosyls in *Aloe*.

The quantitative significance of **1** in 7 *Aloe* species (Table I) was evaluated by HPLC using reversed phase packing ( $C_{18}$ ) and isocratic 50% aqueous MeOH. A baseline separation and determination of the diastereomers **1**, aloins A/B and aloinosides A/B in methanolic leaf extracts succeeds within about 20 min in a single HPLC run [3]; see Fig. 2. The HPLC results demonstrate for the first time that **1** represents a major constituent in *Aloe* leaves and is therefore a useful marker: The content of **1** varies from 0.1 to 6.6% (Table I). It is found in similar amounts as aloins A/B and aloinosides A/B. In *A. khamiensis* and *A. broomii* **1** is the most remarkable anthrone-C-glucosyl. Concerning to our interest in the potential realization of the B-diastereomer of 5-hydroxyaloin, as it was

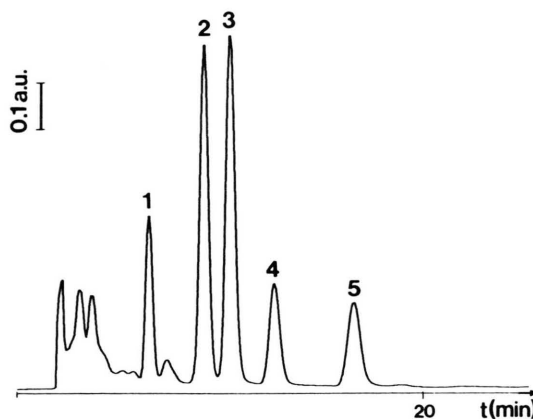


Fig. 2. Reversed phase HPL chromatogram at 360 nm of a methanolic leaf extract from *A. ferox* Mill. Conditions as in Materials and Methods. **1** = 5-hydroxyaloin A, **2** = aloin B, **3** = aloin A, **4** = aloinoside B, **5** = aloinoside A.

demonstrated by  $^1\text{H}$  NMR experiments [14], the HPLC separation confirms that in *Aloe* plants – as in Cape aloes drugs – **1** only occurs in the more stable A-configuration.

In summary, our qualitative and quantitative results corroborate that **1** characterizes a distinguished and quantitatively important hydroxylating pathway in biosynthesis of anthrone-C-glucosyls in *Aloe*. The compound is a possible chemotaxonomic marker for the *Aloe* sections *Pachydendron* and the largest section *Eualoe* which contains about two thirds of all species and is therefore difficult to survey.

#### Acknowledgements

We are grateful to Palmengarten Frankfurt/M. (F.R.G.) and Botanical Garden of Heidelberg (F.R.G.) for supplying plant material. Thanks are also due to Sabine Prodöhl-C.P., Frankfurt/M. (F.R.G.), for providing the leaf extracts for TLC screening.

- [1] H. W. Rauwald, Pharm. Weekbl. Sci. Ed. **9**, 215 (1987).
- [2] H. Böhme and L. Kreutzig, Dtsch. Apoth. Ztg. **103**, 505 (1963).
- [3] H. W. Rauwald and A. Beil, J. Chromatogr. (1993), in press.
- [4] R. K. Mapp and T. J. McCarthy, Planta Med. **18**, 361 (1970).
- [5] H. W. Rauwald, Pharm. Ztg. Wiss. **3**, 169 (1990).
- [6] A. Beil and H. W. Rauwald, in: Hagers Handbuch der Pharmazeutischen Praxis, Vol. 4 (R. Hänsel, K. Keller, H. Rimpler, and G. Schneider, eds.), pp. 209–232, Springer, Berlin 1992.
- [7] T. Reynolds, Bot. J. Linn. Soc. **90**, 157 (1985).
- [8] T. Reynolds, Bot. J. Linn. Soc. **90**, 179 (1985).
- [9] S. Prodöhl-C.P., Ein Beitrag zur chemotaxonomischen Charakterisierung von Aloe-Arten, Diplomarbeit, Universität Frankfurt/M. (1990).
- [10] H. W. Rauwald, Arch. Pharm. (Weinheim) **315**, 769 (1982).
- [11] T. Reynolds and E. Nicholls, Bot. J. Linn. Soc. **92**, 393 (1986).
- [12] H. Jakobsen, Das Sukkulantenlexikon, 2. ed., pp. 57–94, Gustav Fischer, Stuttgart 1981.
- [13] G. W. Reynolds, The Aloes of South Africa, The Aloes of South Africa Book Fund, Johannesburg, South Africa 1950; and The Aloes of Tropical Africa and Madagascar, The Aloes Book Fund, Mbabane, Swaziland 1966.
- [14] H. D. Höltje, K. Stahl, K. Lohse, and H. W. Rauwald, Arch. Pharm. (Weinheim) **324**, 859 (1991).