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

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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]. Refering 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]).

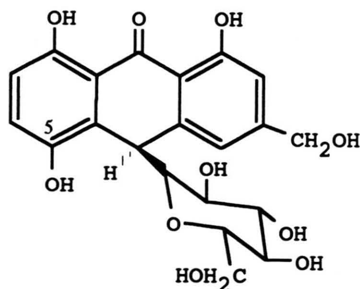

Fig. 1. 5-Hydroxyaloin A.

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

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

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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-

tanical gardens. Extraction and sample preparation were carried out as described by [8]. TLC plates: silica gel UV_{254} (Macherey-Nagel & Co., Düren, F.R.G.); solvent systems: $CHCl_3-MeOH-H_2O$ (7:13:8, lower phase; permits separation of diastereomeric anthrone-C-glucosyls [10]) and $CHCl_3-MeOH$ (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-hydroxyaloins A/B, 7-hydroxyaloin-6′-monoacetates and -4′,6′-diacetates A/B, 8-O-methyl-7-hydroxyaloins A/B and their 6′-cinnamoyl esters) were identified with authentic reference substances which are available in our laboratory. Under UV_{366} 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.

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

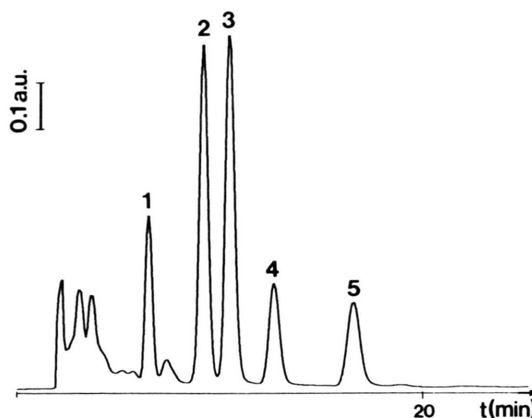

Fig. 2. Reversed phase HPL chromatogram at 360 nm of a methanolic leaf extract from *A. ferox* Mill. Conditions as in Materials and Methods. 1 = 5-hydroxyaloin A, 2 = aloin B, 3 = aloin A, 4 = aloinoside B, 5 = aloinoside A.

demonstrated by ^{1}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.

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