Pergamon

PII: S0031-9422(96)00710-8

Phytochemistry, Vol. 44, No. 7, pp. 1271–1274, 1997

0031-9422/97 $17.00+0.00

ANTHRONES FROM *ALOE MICROSTIGMA*

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(*Received* 28 *August* 1996)

Key Word Index—*Aloe microstigma*; Aloaceae; *C*-glucoside anthrone; 5-hydroxyaloin A; microstigmin A; caffeate; chemotaxonomy.

Abstract—5-Hydroxyaloin A and a new anthrone, named as microstigmin A, were isolated from the leaf exudate of *Aloe microstigma*. The structure of microstigmin A was determined by spectroscopic techniques as well as by conversion into 5-hydroxyaloin A. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

As part of our continuing study of the chemistry of *Aloe* species of Africa [1–3], we have investigated the constituents of the leaf exudate of *A. microstigma* Salm-Dyck, a species endemic to South Africa. We now report the isolation and characterization of the rare anthrone 5-hydroxyaloin A (**1**) as well as of its new natural derivative, 6′-*O*-caffeoyl-5-hydroxyaloin A (**2**), for which the trivial name microstigmin A is suggested. Rauwald and Beil [4] have already demonstrated the presence of **1**, the so-called 'periodate-positive substance' [5], in the leaf exudate of *A. microstigma* by means of TLC and HPLC analysis. The new compound **2** is restricted in its taxonomic distribution to species placed by Reynolds [6] in the *Aloe* series *Purpurascentes* Salm-Dyck (excluding *A. gariepensis* Pillans and *A. succotrina* Lam., but including *A. broomii* Schonl., *A. chlorantha* Lavranos and *A. pictifolia* Hardy). Compound **2** is thus an important chemotaxonomic marker for the series *Purpurascentes* as redefined here. A detailed phylogenetic analysis of the morphology, based on chemical characteristics, is in progress.

RESULTS AND DISCUSSION

Our investigation of the methanol-soluble part of the leaf exudate of *A. microstigma* showed three main constituents on TLC, with R_f values of 0.3, 0.5 and 0.6 (silica gel chloroform–methanol, 4:1). Column chromatography over silica gel with ethyl acetate–methanol gradients followed by further purification by prep. TLC and by column chromatograph, on Sephadex LH-20, eluting with methanol, led to the isolation of the two less polar substances (**1** and **2**). Work on the most polar substance with R_f 0.3 is in progress.

Identification of 5-*hydroxyaloin A* (**1**)

The yellow amorphous compound **1** with R_f 0.5 could be identified as the known 5-hydroxyaloin A according to the ^1H and ^{13}C NMR data (Tables 1 and 2) including the NOESY spectra. The negative-ion FAB mass spectrum exhibited a $[M-H]^-$ ion peak at m/z 433, which is in agreement with $C_{21}H_{22}O_{10}$ for 5-hydroxyaloin A. This compound has been reported earlier to occur only in a few *Aloe* species and is also known as a minor constituent of *A. ferox* Mill., the major source of Cape aloe [7].

Microstigmin A (**2**)

The constituent **2** with R_f 0.6 was obtained as a yellow amorphous solid. Positive-ion FAB mass analysis showed a pseudomolecular ion peak at m/z 597 $[M+H]^+$ indicating a M_r of 596. The molecular formula was found to be $C_{30}H_{28}O_{13}$ by HR mass spectrometry (597.1602 for $[M+H]^+$). In addition, the base peak in the EI mass spectrum at m/z 272 was in agreement with an anthrone moiety. The fragmentation pattern with peaks at m/z 180 $[(HO)_2C_6H_3CH{=}CHCOO]^+$, 163 $[(HO)_2C_6H_3CH{=}CHCO]^+$, 136 $[(HO)_2C_6H_3CH{=}CH+1]^+$, 123 $[(HO)_2C_6H_3CH+1]^+$

Table 1. ^{1}H NMR spectral data for 5-hydroxyaloin A (**1**) (in acetone-d_6) and microstigmin A (**2**) (in methanol–d_4-dimethyl sulphoxide-d_6)

H	1	2
1-OH	11.87 *s*	11.50 *s*
8-OH	11.46 *s*	11.20 *s*
2	6.89 *br s*	6.84 *d*(1.0)
4	7.16 *br s*	7.05 *d*(1.0)
6	6.78 *d*(8.9)	6.77 *d*(8.9)
7	7.18 *d*(8.9)	7.12 *d*(8.9)
10	4.83 *d*(2.1)	4.85 *d*(1.9)
H_2-15	4.69 *s*	4.64 *s*
1′	3.55 *dd*(9.7, 2.1)	3.40 *dd*(9.5, 1.9)
2′	2.88 *dd*(9.7, 9.6)	3.02 *dd*(9.5, 9.4)
3′	3.30	3.34 *t*
4′	3.03	2.98 *t*
5′	3.01	3.14 *ddd*(9.5, 6.9, 1.9)
$6'_a$	3.45 *dd*	3.93 *dd*(11.8, 6.9)
$6'_b$	3.56 *dd*	4.34 *dd*(11.8, 1.9)
2″		7.14 *d*(1.9)
5″		6.87 *d*(8.2)
6″		7.04 *dd*(8.2, 1.9)
7″		7.45 *d*(15.9)
8″		6.20 *d*(15.9)

Coupling constants (J in Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of 5-hydroxyaloin A (**1**) (in acetone-d_6) and microstigmin A (**2**) (in methanol-d_4)

C	1	2
1	162.8	162.8
2	113.6	114.2
3	152.7	152.0
4	117.6	117.5
5	147.0	147.3[a]
6	117.3	117.6
7	125.3	124.6
8	156.8	156.9
9	195.3	195.4
10	40.9	40.6
11	118.5	119.2
12	126.9	126.4
13	117.1	118.2
14	145.4	146.4
15	64.1	64.5
1′	84.4	85.8
2′	72.8	73.0
3′	78.9	78.6
4′	72.0	71.9
5′	81.0	79.0
6′	63.3	64.8
1″		127.9
2″		115.6
3″		146.7[a]
4″		149.5
5″		116.4
6″		123.0
7″		146.9
8″		115.0
9″		169.0

[a] Assignments may be interchanged in this column.
Signal assignments are based on ^{1}H–^{13}C COSY spectrum.

and 110 $[(HO)_2C_6H_3+1]^+$ suggested a caffeate unit as part of the molecule.

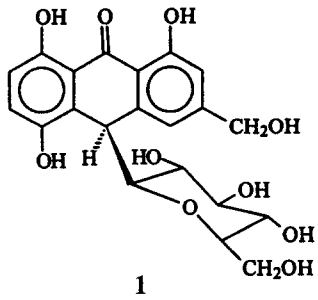
The IR spectrum of **2** exhibited absorptions consistent with a hydroxyl (3390 cm^{-1}), a conjugated ester carbonyl (1685 cm^{-1}) and a chelated carbonyl group (1637 cm^{-1}). The ^{13}C NMR and DEPT spectra showed signals for 30 different carbon atoms corresponding to two oxymethylenes, 15 methines and 13 quaternary carbon atoms including one chelated carbonyl (δ 195.4) and one ester carbonyl carbon (δ 169.0). Further evidence was obtained from the ^{1}H NMR spectrum of **2**, which showed signals comparable to those of **1**: oxymethylene protons H_2-15 (δ 4.64, *s*), a methine proton H-10 (δ 4.85, *d*, J = 1.9 Hz), two *meta*-coupled aromatic protons H-2 (δ 6.84, *d*, J = 1.0 Hz) and H-4 (δ 7.05, *d*, J = 1.0 Hz) as well as two *ortho*-coupled aromatic protons H-6 (δ 6.77, *d*, J = 8.9 Hz) and H-7 (δ 7.12, *d*, J = 8.9 Hz). The downfield shift of the signals corresponding to the C-6′-methylene protons in **2** (from δ 3.45 and 3.58 in **1** to δ 3.93 and 4.34 in **2**) is in agreement with the esterification of this hydroxyl group. Analysis of the signals for protons assignable to two *trans*-vinyl H-8″ (δ 6.20, *d*, J = 15.9 Hz) and H-7″ (δ 7.45, *d*, J = 15.9 Hz) and to three aromatic protons H-2″ (δ 7.14, *d*, J = 1.9 Hz), H-5″ (δ 6.87, *d*, J = 8.2 Hz) and H-6″ (δ 7.04, *dd*, J = 8.2 and 1.9 Hz) led to a *trans*-caffeoyl residue as carbonyl part.

Compound **2** was hydrolysed under acidic conditions to give **1**. Important observations from the NOESY and ^{1}H–^{1}H COSY can be summarized as follows (see also Fig. 1): (i) cross peaks between the methine proton H-10 (δ 4.85, *d*) with H-1′ (δ 3.40, *dd*) of the glucose moiety and with only one of the aromatic protons H-4 (δ 7.05, *d*); (ii) cross peaks of the oxymethylene protons H_2-15 (δ 4.64, *s*) with two aromatic protons H-2 (δ 6.84, *d*) and H-4 (δ 7.05, *d*); (iii) *ortho* coupling between the two aromatic protons H-6 (δ 6.77, *d*) and H-7 (δ 7.12, *d*).

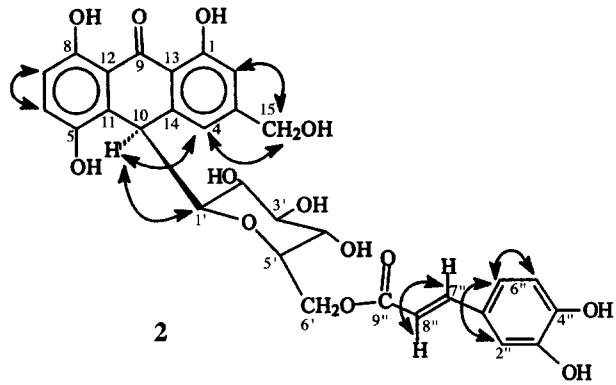
Rauwald and Beil have already demonstrated, that **1** naturally exists only in the more stable A form, in which the glucose moiety has a β-orientation at C-10 [4]. This is in contrast to aloin, which is found as a mixture of the A and the B form. Indeed, for **1** we do not observe signal doubling in the ^{1}H and ^{13}C NMR spectra or other characteristics (e.g. two spots on TLC) which are typical of aloe anthrones existing as diastereomeric pairs. Since the circular dichroic spectra (Fig. 2) of **1** and **2** are very similar, the β-orientation can be deduced also for the glucose moiety at C-10 in **2**. All of the above data are in agreement with structure **2** for the new compound: 6′-*O*-*trans*-caffeoyl-5-hydroxyaloin A, named as microstigmin A.

EXPERIMENTAL

General. Mps: uncorr. Optical rotation in MeOH; UV in MeOH; IR: KBr discs; ^{1}H and ^{13}C NMR



1



2

Fig. 1. NOESY and ^1H–^1H correlations of compound **2**.

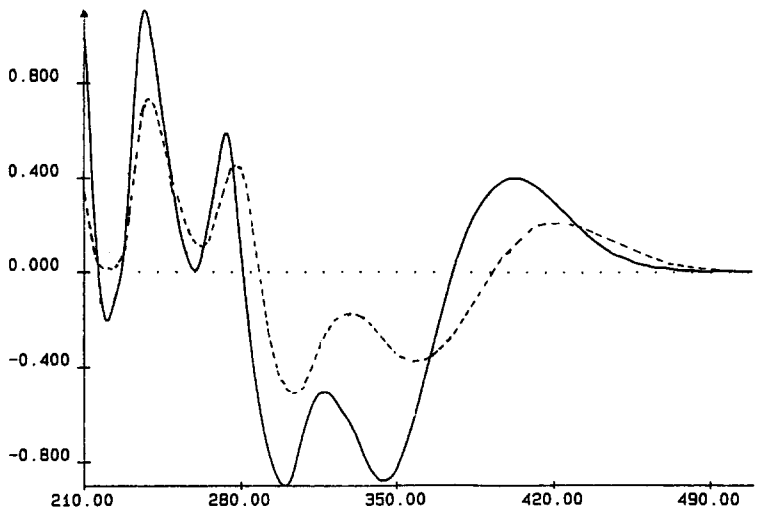


Fig. 2. CD spectra in methanol of microstigmin A (——) and 5-hydroxyaloin A (– – –).

(Bruker ARX 300, 300 and 75 MHZ, respectively) in Me_2CO-d_6 or MeOH-d_4 with the solvent as int. standard; FAB-MS (Finnigan MAT 90 or Finnigan MAT 95Q double focusing instrument with Cs gun): *m*-NBA-matrices; TLC solvent system on silica gel: I($CHCl_3$–MeOH, 4:1).

Plant material. A bulk sample of leaf exudate of *A. microstigma* was collected from a natural population near Worcester in the Western Cape Province of South Africa and identified by B.-E. Van Wyk.

Extraction and isolation. Leaf exudate of *A. microstigma* (10 g) was taken up in MeOH. The MeOH extract was concd and the residue was subjected to flash CC over silica gel eluting with EtOAc and MeOH gradients. The frs were further purified by applying to prep. TLC plates and Sephadex LH-20 (MeOH), which resulted in isolation of pale yellow substances: **1** (30 mg) and **2** (200 mg), respectively.

5-Hydroxyaloin A (**1**). Yellow amorphous solid. $[\alpha]_D$ −70° (MeOH; *c* 1.0). R_f 0.5 (solvent I). UV λ_{max} nm:

271, 298, 355. IR ν_{max} cm^{-1}: 3425, 1634, 1601, 1447, 1380, 1273. ^1H NMR (Table 1) and ^{13}C NMR (Table 2). Negative-ion FAB-MS: *m/z* 433 $[M-H]^-$; positive-ion FAB-MS: *m/z* 419 $[M-OH+H]^+$.

Microstigmin A (**2**). Yellow solid, mp 152–154°. $[\alpha]_D$ −20° (MeOH; *c* 1.0). R_f 0.6 (solvent I). UV λ_{max} nm: 245, 299, 329. IR ν_{max} cm^{-1}: 3390, 1685, 1637, 1607, 1465, 1376, 1273, 1185. ^1H NMR (Table 1) and ^{13}C NMR (Table 2). Negative-ion FAB-MS: *m/z* 595 $[M-H]^-$; positive-ion FAB-MS: *m/z* 619 $[M+Na]^+$, 597 $[M+H]^+$, HR-MS: 597.1602, calc. 597.1608; EIMS *m/z* (rel. int.): 596 (9), 323 (22), 309 (38), 295 (47), 272 (100), 180 (25), 163 (16), 136 (66), 123 (23), 110 (13).

Acid hydrolysis of **2**. A soln of **2** (10 mg) in 1% methanolic HCl (2 ml) was stirred for 6 hr at room temp. After removal of solvent, the reaction mixt. was neutralized with 10% $NaHCO_3$ and extracted with $CHCl_3$ to give a product (3 mg) identical to **1** (co-TLC and ^1H NMR).

Acknowledgements—E. D. is grateful to SAREC (Sweden) for a research grant, D. B. is grateful to IPICS-NAPRECA for a Home University-based training fellowship, and B.-E. V. W. and A. M. V. wish to thank the South African Foundation for Research Development for financial support.

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