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CONSTITUENTS OF THE LEAVES OF FOUR *ALOE SPECIES* FROM ETHIOPIA

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ABSTRACT. Chemical investigation of the leaves of *Aloe berhana*, *A. rivae*, *A. megalacantha* and *A. pulcherima* resulted in the isolation of chrysophanol, β-sitosterol, aloe-emodin, aloechrysone, protocatechuic acid, barbaloin, aloinoside, nataloin and 7-hydroxybarbaloin. The results presented in this study indicate that these species compare favourably with the well known aloes of commerce.

INTRODUCTION

The bitter leaf exudates or latex of many *Aloe species* are known to have various medicinal uses [1] and are used in the preparation of different types of drugs including the laxative aloe drug [2].

A. berhana Reynolds is an endemic species widely distributed in the central highlands of Ethiopia particularly on the Addis Ababa - Debre Berhan road. *A. rivae* Baker and *A. megalacantha* Baker are also known to occur in Kenya and Somalia, respectively [3]. *A. pulcherima* Gilbert and Sebsebe (ined) was only recently described [4], and is believed to be an endemic species to the area around Addis Ababa in central Ethiopia. This species is distinct because of the lack of spines on the leaf margins, a feature that is rather unusual for *Aloe* species.

We wish to report here our results of the chemical investigation of the leaves of the above four species. Except for an HPLC screening of barbaloin in *A. rivae* and *A. megalacantha* by Groom and Reynolds [5], there is no phytochemical report on the above species.

RESULTS AND DISCUSSION

In all cases dried and powdered leaves were extracted with EtOH and the extract subjected to CC over silica gel. This resulted in the isolation and characterization of nine compounds. Chrysophanol (**1**) and β-sitosterol (**2**) were isolated from all four species. Aloe-emodin (**3**) and barbaloin (**6**) were obtained from *A. berhana*, *A. rivae* and *A. megalacantha*. These three species also yielded the preanthraquinone, aloechrysone (**4**), a novel compound that we recently discovered in the roots of *A. berhana* [6].

Furthermore aloinoside (**7**) was obtained from *A. rivae* and *A. megalacantha*, while 3,4-dihydroxybenzoic acid or protocatechuic acid (**5**) was isolated from *A. berhana*. The recently described species *A. pulcherima* additionally yielded nataloin (**8**) and 7-hydroxybarbaloin (**9**).

The identification of the above compounds was mainly based on spectral data, in some cases comparison with authentic samples, chemical transformation

and degradation to known compounds. Compounds **1, 2, 4, 5, 6** and **8** were characterized by comparison of their physical and spectral data with those reported in the literature (see Experimental section).

Compounds **7** and **9** were elucidated based on comparison with the spectral data of **6** and **8**. ^{1}H and ^{13}C NMR data of these four glycosides (**6-9**) are given in Tables 1 and 2, respectively. This is the first report of ^{13}C data for **7** and **9**. The three C-glycosides **6, 8** and **9** were also subjected to $FeCl_3$ oxidative hydrolysis [2], and **7** to acid hydrolysis, from which the aglycones and sugar moieties were identified.

Table 1. ^{1}H NMR data of compounds **6, 7, 8** and **9**.

H	6	8	9	7
2	6.8 brs	6.8 brs	6.8 brs	6.8 brs
4	7.0 brs	6.6 brs	6.6 brs	7.0 brs
5	6.9 d(7.8)	7.0 d(7.6)	7.0 d(7.6)	6.9 t(7.8)
6	7.5 t(7.8)	6.85 d(7.6)	6.85 d(7.6)	7.4 t(7.8)
7	7.1 d(7.8)	-	-	7.1 d(7.8)
10	4.6 brs	4.4 brs	4.4 brs	4.6 brs
3-CH_2	4.6 brs	-	4.8 brs	4.8 brs
3-CH_3	-	2.4 s	-	-
1,8-OH	11.85, 11.8	11.8, 11.7[a]	11.8, 11.75	11.8 brs
7-OH	-	8.85[a]	8.75	-
1'	3.2 brs	3.2 brs	3.1 brs	3.3 brs
2'-6'	2.5-3.4 m	2.7-3.5 m	2.8-3.3 m	2.5-3.4 m
1"	-	-	-	5.1 brs
2"-5"	-	-	-	3.5-4.9 m
6"	-	-	-	1.2 d(4.5)

spectra run in DMSO-d_6 at 90 MHz, J values in parentheses
a = exchangeable with D_2O.

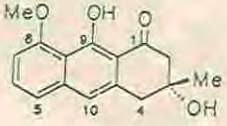
Table 2. ^{13}C NMR data of compounds **6, 7, 8** and **9**.

C	6	8	9	7
1	160.7	160.7	160.8	160.8
2	112.5	120.9	121.3	113.7
3	151.3	149.3	149.4	145.3
4	117.6	115.3	112.5	117.1
5	119.8	117.7	117.6	118.0
6	135.5	121.4	120.5	135.4
7	115.3	144.0	145.0	115.3
8	160.7	146.1	146.0	160.8
9	193.6	193.6	193.7	193.2
10	43.9	43.6	43.4	43.9
11	115.0	120.5	119.1	114.0
12	141.5	134.4	135.0	141.3
13	145.2	142.4	143.0	145.2
14	117.0	117.7	117.1	117.0
15	62.3	21.3	62.4	67.0
1'	79.9	78.7	78.1	78.0
2'	70.4	70.5	70.4	70.4
3'	80.2	80.1	80.1	79.5
4'	70.1	70.1	70.1	70.1
5'	84.8	84.7	84.7	84.7
6'	61.5	61.7	61.6	61.7
1"	-	-	-	99.4
2"	-	-	-	70.8
3"	-	-	-	72.4
4"	-	-		68.5
5"	-	-	-	84.7
6"	-	-	-	17.1

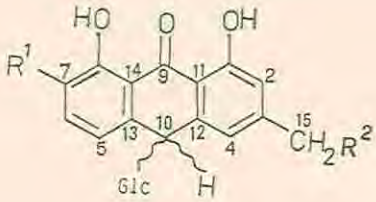
We note that the above four *Aloe* species compare favourably with the well known aloes of commerce. Thus barbaloin (**6**), which represents the bitter and purgative principle of the drug aloe [7], is present in good yield in *A. berhana*,

A. rivae and *A. megalacantha*. Aloinoside (**7**) which is one of the principal constituents of Socotrine and Cape aloes [8] is also to be found in *A. rivae* and *A. megalacantha*.

A. pulcherima, is known in some localities in Shoa province of Ethiopia as "Sete Eret" and various types of medicinal uses are ascribed to it in folk medicine. It is interesting to note that barbaloin does not occur in its leaves. Its major components are nataloin (**8**) and 7-hydroxybarbaloin (**9**). Compound **8** is an important constituent of Natal aloes [9] while **9** is the characteristic substance of Curacao aloes [10].



4



	R^1	R^2
6	H	OH
7	H	O-Rham
8	OH	H
9	OH	OH

The isolation of the simple biologically important aromatic acid, **5**, from *A. berhana* is noteworthy. We have confirmed that this compound is not an artifact, since two different extraction methods namely the one employed in lit. [9], fresh leaves extd with MeOH followed by partitioning with EtOAc, and that described in this report, have resulted in the isolation of this compound. Although compound **5** is rare from a natural source, it has been reported to occur in coffee pulp [11] and can also be extracted from lignin [12], however this is the first report of its existence in an *Aloe* species.

EXPERIMENTAL

General. Mps are uncorr. ^1H and ^{13}C NMR were measured at 90 and 22.5 MHz, respectively, on Jeol FX90Q NMR spectrometer. IR spectra were recorded as KBr discs. UV spectra were recorded in MeOH solutions.

Plant materials. The leaves of *A. berhana* were collected from 9 km SW of Debre Berhan, ca. 9°40'N 39°40'E; *A. rivae* from Mega, alt. 2201 m; *A. megalacantha* from Asbe Teferi, alt. 1830 m; and *A. pulcherima* from Debre Libanos, alt. 2470 m. Voucher specimens, Sebsebe 2209, 2201, 2282 and 2386, respectively, are kept at the National Herbarium, Addis Ababa University.

Extraction and isolation of compounds. The powdered leaves of *A. berhana* (85 g), *A. rivae* (185), *A. megalacantha* (200) and *A. pulcherima* (300) were separately extd with EtOH in a Soxhlet apparatus each for 12 hrs. Removal

of EtOH from each yielded 5.2, 10, 18 and 28 g of the corresponding dark oily residue. The crude exts were then subjected to CC over silica gel 60 and elution was carried out in each case using petrol-EtOAc followed by EtOAc-MeOH mixtures of increasing polarities. Column fractions were analysed by TLC and purified accordingly by Sephadex LH-20, recrystallization and PTLC. Compounds **1** and **2** were obtained from all four upon elution with petrol-EtOAc (9:1 and 7:3), respectively. The petrol-EtOAc (6:4) eluate of *A. berhana*, *A. rivae* and *A. megalacantha* yielded **3** and **4**. Compound **5** was obtained only from *A. berhana* using petrol-EtOAc (1:1) as an eluent. From the EtOAc-MeOH (9:1) eluate of *A. berhana*, A. rivae and *A. megalacantha* was isolated **6** (20, 900 and 850 mg, respectively), while from that of *A. pulcherima* was obtained 3 (510 mg). Finally elution of *A. rivae* and *A. megalacantha* with EtOAc-MeOH (2:1) afforded **7** (455 and 255 mg, respectively) and of *A. pulcherima yielded* **9** *(300 mg).*

Chrysophanol (**1**). Orange needles from MeOH, mp 193-195° (lit. [13] 197°); UV and IR in good agreement with lit. [13].

β-Sitosterol (**2**). White needles from Me_2CO, mp 137° (lit. [12] 136°). Found $[M]^+$ 414.3892, $C_{29}H_{50}O$ requires 414.3861. ^1H and ^{13}C NMR correlated well with those published for sitosterol 3-O-glycosides [14]. EIMS m/z (rel. int.): 414 $[M]^+$ (100), 399 $[M-Me-18]^+$ (37), 396 $[M-H_2O]^+$ (50), 381 (37), 329 (38), 273 (27), 255 (33), 231 (23).

Aloe-emodin (**3**). Orange-yellow needles from MeOH, mp 218-220° (lit. [8] 223-224). Obtained in trace quantities and identified by comparison with authentic sample.

Aloechrysone (**4**). Greenish-yellow pigment, oily; $[\alpha]_D$ -4 ($CHCl_3$, c 0.02); ^1H NMR ($CDCl_3$) δ: 1.5 (3H, s, Me), 2.8 (2H, s, H-2), 3.2 (2H, s, H-4), 4.0 (3H, s, OMe), 6.8 (1H, d, J=7.6 Hz, H-7), 7.0 (1H, s, H-10), 7.2 (1H, d, J=7.6 Hz, H-5), 7.5 (1H, t, J=7.6 Hz, H-6), 15.0 (1H, s, OH-9). UV identical to lit. [6].

Protocatechuic acid (**5**). Brown amorphous solid, mp 190° (lit. [15] 200-202°). Found $[M]^+$ 154.0264; $C_7H_6O_4$ requires 154.0266. IR ν_{max} cm^{-1}: 3300-3200, 1700, 1650-1400, 1250. UV λ_{max} nm: 206, 257, 293. ^1H NMR (DMSO-d_6)δ: 6.7 (1H, d, J=8 Hz, H-5), 7.3 (1H, dd, J=8, 2, H-6), 7.4 (1H, d, J=2, H-2). ^{13}C NMR identical to lit. [16]. EIMS m/z (rel. int.): 154 $[M]^+$ (100), 137 $[M-OH]^+$ (100), 109 $[M-CO_2H]^+$ (47).

Barbaloin (**6**). Yellow needles from EtOH, mp 140-144° (lit. [12] 146-148°). $[\alpha]_D$ 0 (MeOH, c 0.02); UV identical to lit. [17]. IR ν_{max} cm^{-1}: 3400, 2950, 1630, 1480, 1385, 1285. ^1H and ^{13}C NMR see Table 1 and 2, respectively. FABMS m/z (rel. int.): 419 $[M+H]^+$ (51), 256 (55), 239 (63), 227 (13), 153 (25).

Aloinoside (**7**). Light yellow amorphous solid, mp 228-231° (lit. [8] 233°). $[\alpha]_D$ +75 (MeOH, c 0.02); IR ν_{max} cm^{-1}: 3450, 3250, 1630, 1460, 1385, 1280. ^1H and ^{13}C NMR see Table 1 and 2. FABMS m/z (rel. int.): 565 $[M+H]^+$ (32), 419 (22), 402 (12), 256 (10), 239 (63).

Nataloin (**8**). Orange amorphous solid, mp 175-179° (lit. [9] 184-187°). UV identical to lit. [9]. ^1H and ^{13}C NMR see Table 1 and 2, respectively. FABMS m/z (rel. int.): 419 $[M+H]^+$ (36), 256 (57), 227 (9), 152 (55).

7-Hydroxybarbaloin (**9**). Yellow amorphous solid, mp 125-128° dec. UV λ_{max} nm: 268, 304, 358. IR ν_{max} cm^{-1}: 3740-3400, 3000, 1625, 1600, 1460. ^1H and ^{13}C NMR see Table 1 and 2. FABMS m/z (rel. int.): 435 $[M+H]^+$ (78), 272 (100), 255 (80), 244 (12).

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