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ANTHRONES FROM *ALOE BARBADENSIS*

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Key Word Index—*Aloe barbadensis*; Liliaceae; root; leaf sap; anthrones; 2D-NMR.

Abstract—A new compound, 3,4-dihydro-3,5,7-trihydroxy-9-methyl-1(2H)-anthracenone, designated as aloe barbendol has been isolated from the roots of *Aloe barbadensis*, while three known constituents, aloe emodin, aloe chrysone and barbaloin A have been obtained from the sap of the leaves. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Aloe barbadensis known in the vernacular as 'Ghikunvar', is a short-stemmed succulent herb which is widely distributed in Asia, Africa and other tropical parts of the world [1, 2]. It has a very ancient history for healing wounds and also finds use in cosmetics [3, 4]. Its leaves are well reputed in folk medicine for the treatment of asthma, gastrointestinal ulcers, burns and chronic wounds [1–4]. Biological studies revealed that the leaves of this species possess purgative, analgesic, antipyretic, anticancer [2, 5], antimicrobial [6], immunological adjuvant [7] and insecticidal activities [2]. Chemical constituents present in *A. barbadensis* are mostly anthraquinones, chromones and polysaccharides [7–10], which are also responsible for its antiviral, immunological adjuvant and wound healing properties [7, 10, 11].

Natural products isolated so far from *A. barbadensis* are barbaloin [8, 12], tetranitroaloe emodin [13], aloesin, isoaloesin [9], β-sitosterol [14], gibberellins [15], polysaccharides, including carrisyn [10] and aloeferon [16], and some alkanes, fatty acids and alkyl benzenes [17]. Most of the chemical work is confined to its leaves and no scientific data is available on the roots. In the genus *Aloe*, which comprises over 360 species, isolation of chemical constituents of roots is limited to only four species, *A. saponaria* [18], *A. acutissima* [19], *A. berhana* [20] and *A. graminicola* [21]. Recently two reports were published in which anthranoids in roots of different *Aloe* species had been detected chemotaxonomically [21, 22].

In the present work, a fresh root extract of *A. barbadensis* was subjected to vacuum liquid chromatography (VLC) and flash column chromatography (FCC) which resulted in the isolation of a new anthracenone, aloe barbendol (**1**). In addition to this, three known constituents, aloe emodin (**2**) [23], aloe chrysone (**3**) [20] and barbaloin A (**4**) [8, 12] were isolated from the fresh sap of its leaves, employing classical method of isolation followed by VLC. This is the first report of the isolation of aloe emodin, which is a potent antiviral [11], antimutagenic [5] and tuberculostatic [24] agent, and aloe chrysone from this source. Aloe emodin is usually prepared from barbaloin [11], which is the most abundant compound of *A. barbadensis* [25], whereas aloe chrysone was isolated earlier from the roots of *A. berhana* [20]. The first naturally occurring *C*-glycoside, barbaloin A (**4**) [25], which could be separated from its stereoisomeric B form only by HPLC or DCCC [8] has now been obtained by classical methods of isolation and VLC. Structures of all of these compounds have been determined through UV, IR, mass, ^{1}H and ^{13}C NMR spectroscopy and appropriate 2D-NMR (COSY, NOESY, HMQC, HMBC and *J*-resolved) techniques. Spectral data of known compounds are comparable with those reported in literature [8, 18, 20, 23] and with those of authentic sample.

RESULTS AND DISCUSSION

A methanolic extract of fresh roots of *A. barbadensis* on subjecting to VLC and FCC afforded

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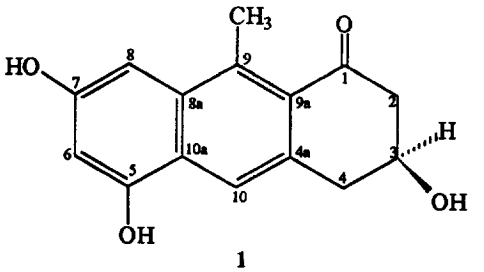

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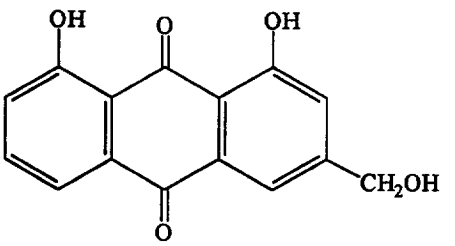

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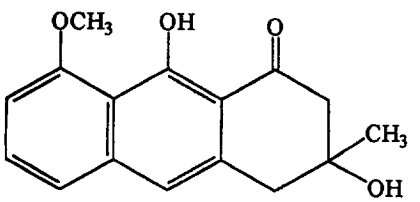

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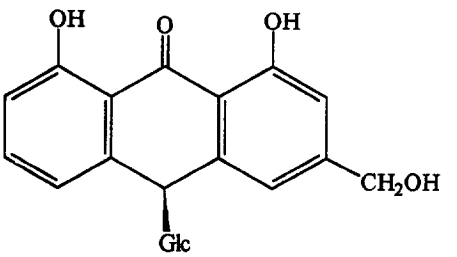

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many fractions, one of which appeared as a single blue fluorescent spot on TLC when viewed at 366 nm. This spot was identified subsequently as a new compound, aloe barbendol (**1**).

The molecular formula of **1** was established as $C_{15}H_{14}O_4$ by exact mass measurement of the $[M]^+$ at *m/z* 258.0880 in the EI mass spectrum. The broad band ^{1}H-decoupled ^{13}C NMR (BB) and distortionless enhancement by polarization transfer (DEPT) spectra indicated that the 15 carbons in the molecule are present as one methyl, two methylene, one methine, three sp^2 CH and eight sp^2 quaternary carbons. The structural features of **1** were portrayed as 3,4-dihydroanthracenone by virtue of the similarity of its UV and ^{1}H NMR spectral data to those of aloe saponol II [18]. The ^{1}H NMR spectrum in MeOH-d_4 at 400 MHz exhibited in the aromatic region a one-proton singlet at δ 6.85 (H-10), a one-proton doublet at δ 6.76 ($J_{6,8}$ = 2.5 Hz, H-6) and a one-proton broad doublet at δ 6.74 ($J_{8,6}$ = 2.5 Hz, H-8) showing their connectivities with respective carbons at δ 118.25, 108.66 and 120.71 in the HMQC plot. A three-proton singlet at δ 2.84 in the ^{1}H NMR spectrum and a peak at δ 24.9 in the ^{13}C NMR spectrum showed the presence of a methyl group in the molecule. Its placement at C-9 was determined by NOESY and HMBC experiments (Fig. 1). Nuclear Overhauser spectroscopy (NOESY) displayed a stereo linkage between H-8 (δ 6.74) and 9-CH_3 (δ 2.84), while in HMBC, long-range relations were observed between methyl protons (δ 2.84) and C-9 (δ 143.59) and C-8 (δ 120.71); H-8 (δ 6.74) and methyl carbon (δ 24.93) and C-6 (δ 108.66). Long-range coupling of H-8 with methyl protons further confirmed the position of the methyl group at C-9. The presence of aliphatic protons constituting ring C of the molecule, was also evidenced by the ^{1}H NMR spectrum as it showed a pair of one-proton doublet of double doublets at δ 3.19 (J_{gem} = 15.6 Hz, $J_{4e,3}$ = 3.6 Hz, $J_{4e,2}$ = 1.0 Hz, H-4e) and 2.72 (J_{gem} = 13.2 Hz, $J_{2a,3}$ = 7.6 Hz, $J_{2a,4}$ = 1.0 Hz, H-2a), two one-proton multiplets at δ 2.97 (H-4a) and 2.93 (H-2e) and a one-proton triplet of triplets at δ 4.32 ($J_{3,2a=3,4a}$ = 7.6 Hz, $J_{3,2e=3,4e}$ = 3.6 Hz, H-3). An HMQC experiment determined the attachment of the methylene protons at δ 2.72, 2.93 (H-2) with δ_C 47.7, the methine proton at δ 4.32 with δ_C 66.9 and methylene protons at δ 3.19, 2.97 (H-4) with δ_C 39.2. The methine proton at δ 4.32 indicated the presence of the hydroxyl group at C-3, the exact position of which was assigned from a ^{1}H-^{1}H-COSY-45 spectrum, which

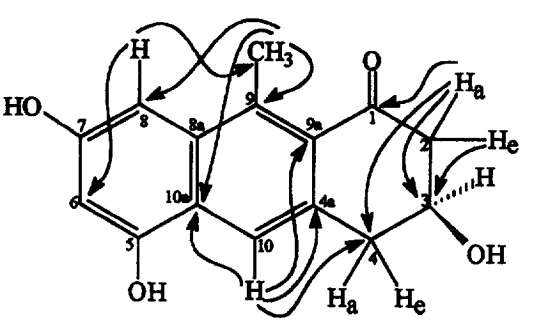

Fig. 1. HMBC connectivities of aloe barbendol (**1**).

showed through-bond connectivity of δ 4.32 (H-3) with 3.19, 2.97 (H-4) and 2.93, 2.72 (H-2). The coupling constant of H-3 revealed it to be axially-oriented. The fact that the non-chelated carbonyl group is located in ring C of the molecule, was determined by the chemical shift of C-1 at δ 202.8 in the ^{13}C NMR spectrum [20], absorption at 1651 cm^{-1} in the IR spectrum and long-range $^{1}H,^{13}C$–correlation in the HMBC plot, which showed a cross-peak for the H-2 proton (δ 2.72) and the carbonyl carbon at C-1 (Fig. 1).

The above chemical shift values are comparable with those reported for 3,4-dihydro-1(2H)-anthracenone in aloe saponol I and II [18, 20], suggesting that aloe barbendol (**1**) also has the 3,4-dihydro-1(2H)-anthracenone skeleton. It is important to note that the chemical shift values for C-2 (47.71) and C-4 (39.16) in **1** are the reverse of those reported for these carbons in aloe saponol I [20]. However, the present assignment is strengthened by the HMBC experiment, showing long-range coupling of H-10 (δ 6.85) with C-4 (δ 39.2) which, in turn, has its connectivity with H-4 (δ 3.19, 2.97) in the HMQC spectrum. Assignment of all the quaternary carbons were made by HMBC. In light of the above discussion, the structure of aloe barbendol has been elucidated as 3,4-dihydro-3,5,7-trihydroxy-9-methyl-1(2H)-anthracenone (**1**), which was corroborated by the mass fragments at *m/z* 241 $[M-17]^+$, 240.0765 $[M-18]^+$, 225 $[M-18-15]^+$, 205 $[M-18-18-17]^+$, 197 $[M-28-18-15]^+$ and 137.0556 ($C_8H_9O_2$) in the EI mass spectrum. It is therefore a positional isomer of aloe saponol II which was isolated earlier as the first tetrahydroanthracene homologue from *A. saponaria* [18]. The two isomers differ in the position of their methyl and hydroxyl substituents in the aromatic region of the molecule. The presence of non-chelated carbonyl absorption in the IR spectrum and the absence of any chelated hydroxyl proton in the ^{1}H NMR spectrum run in $CDCl_3$, confirmed that instead of having methyl and hydroxyl substituents at C-8 and C-9, respectively, as in aloe saponol II [18, 20], **1** has these groups at C-9 and C-7, respectively.

EXPERIMENTAL

General. UV: MeOH. IR: $CHCl_3$. EIMS: 80 eV. FABMS: negative ionization and positive ionization, 8kV. HRMS: 70 eV. ^{1}H and ^{13}C NMR: 400 and 100 MHz, respectively, in MeOH-d_4 and $CDCl_3$ as solvent with spectra referenced to residual solvent signals. Unambiguous assignments of proton and carbon chemical shifts were made partly through COSY-45, NOESY, DEPT, HMQC and HMBC and partly by comparison with published data of similar compounds [8, 18, 20, 23] and a spectral study of an authentic sample of barbaloin A (Sigma). The purity of compounds was checked by TLC on silica gel 60 GF_{254}.

Plant material. Leaves and roots of *A. barbadensis* Mill. (syn. *A. vera* Tourn. ex Linn.) were collected from the Karachi region in January and March 1996, respectively. A voucher specimen (A. Husain s.n. KUH) is deposited in the Department of Botany, University of Karachi.

Extraction and isolation of aloe barbendol (**1**) *from roots.* Fresh undried roots (1 kg) were cut into small pieces and extracted $\times 4$ with MeOH at room temp. Extracts were combined and solvent removed under red. pres. to give a residue (51.4 g), which was subjected to VLC (Kieselgel 60 PF_{254}: petrol, EtOAc and MeOH in order of increasing polarity by 10%) [26]. The vol. eluted with each fr. was 1 l; 21 frs were ultimately obtained. Six of these frs (petrol–EtOAc, 6:4–9:1) showing the same spots on TLC (silica gel, $CHCl_3$–MeOH, 7:3) were combined and evapd under red. pres. to give a residue (890 mg). This residue was further chromatographed by FCC silica gel 60 (9385), petrol, EtOAc and MeOH in order of increasing polarity [27], which gave many frs. One of these frs. petrol–EtOAc (7.8:2.2) showed a single blue fluorescent spot on TLC (silica gel, benzene–EtOAc, 1:1, R_f 0.37) which was characterized ultimately as aloe barbendol (**1**, 8.76 mg).

Isolation of aloe emodin, aloe chrysone and barbaloin A from leaf sap. Fresh leaves (25 kg) were cut longitudinally at their base and the yellow sap allowed to exude. This was collected and dissolved in MeOH and concd under red. pres. to give a thickish mass (18.5 g), which was partitioned between EtOAc and H_2O. The EtOAc phase, after usual work-up, was freed of solvent to give a residue (3.8 g), which was divided into petrol soluble and insoluble portions. The latter fr. was treated with 80% aq. MeOH and benzene–petrol (1:1), and the layers sepd. The 80% MeOH phase was extracted with EtOAc after satn with NaCl. The residue (2.6 g) obtained on usual work-up of this EtOAc phase was subjected to VLC (Kieselgel 60 PF_{254} petrol, EtOAc and MeOH in order of increasing polarity) which afforded many frs. Three of these frs showed a single spot on TLC. Fr.-1 (petrol–EtOAc, 8:2, 3.2 mg) and fr.-2 (petrol–EtOAc, 7:3, 3.9 mg) showing yellow and greenish fluorescence, respectively, on TLC at 366 nm were identified as aloe emodin (**2**) and aloe chrysone (**3**) with R_f 0.75 and 0.55 (petrol–EtOAc, 1:1), respectively. Fr.-3 (EtOAc–MeOH, 9:1) gave fine yellow needles [mp 147–148° (lit. [13] 148°), $CHCl_3$–MeOH, 1:1, 15.7 mg] which showed a yellow spot on TLC (EtOAc–MeOH–H_2O, 10:2:1, R_f 0.52) and was identified as barbaloin A.

Aloe barbendol (**1**). $[\alpha]_D+56°$ (MeOH; *c* 088). UV λ_{max}^{MeOH} nm: 201.8, 273.8, 380.2. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 1651, 1600, 1590, 1450. EIMS *m/z* (rel. int.) 258.0880 $[M]^+$ ($C_{15}H_{14}O_4$ requires 258.0892) (25), 240.0765 $[M-18]^+$ ($C_{15}H_{12}O_3$) (22), 214 (5), 212 (4), 205 (3), 197 (7), 180 (10), 137.0556 ($C_8H_9O_2$) (21). ^{1}H and ^{13}C NMR in Table 1.

Table 1. ^{1}H and ^{13}C NMR chemical shifts (δ) and coupling constants (Hz) for aloe barbendol (**1**) in methanol-d_4

^{1}H	δ	^{13}C	δ
2a	2.72 *ddd* (13.2, 7.6, 1.0)	1	202.8
2e	2.93 *m*	2	47.7
3	4.32 *tt* (7.6, 3.6)	3	66.9
4a	2.97 *m*	4	39.2
4e	3.19 *ddd* (15.6, 3.6, 1.0)	4a	136.7
6	6.76 *d* (2.5)	5	160.3*
8	6.74 *br d* (2.5)	6	108.7
10	6.85 *s*	7	160.2*
9-CH_3	2.84 *s*	8	120.7
		8a	115.6
		9	143.6
		9a	111.1
		10	118.3
		10a	142.7
		9-CH_3	24.9

* Chemical shifts may be interchanged.

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