FULL PAPER

Aloeverasides A and B: Two Bioactive *C*-Glucosyl Chromones from *Aloe vera* Resin

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Two new *C*-glucosyl chromones named aloeverasides A (**1**) and B (**2**) were isolated from the resin of *Aloe vera* (L.) Burm.f. The structures of the two new natural products were elucidated based on 1D- (^{1}H- and ^{13}C-NMR) and 2D-NMR (COSY, HSQC, and HMBC) spectroscopic techniques and mass spectrometry (ESI-MS). Aloeverasides A (**1**) and B (**2**) were evaluated for their anticancer activity, and both induced a 76.4 and 70.5% growth inhibition of the breast cancer cell line (MDA-MB-231) at a concentration of 100 μM. Both compounds were also evaluated for their 1,1-diphenyl-2-picryl-hydrazyl antioxidant, urease enzyme, and α-glucosidase enzyme inhibition activities. Aloeverasides A (**1**) and B (**2**) displayed good urease enzyme inhibition activities (62 and 55%, resp.), as well as antioxidant activity in which aloeveraside A (**1**) had a value of 60% inhibition, while aloeveraside B (**2**) demonstrated a more potent antioxidant activity with 80% inhibition.

Keywords: *Aloe vera* (L.) Burm.f., Natural products, Chromones, Structure elucidation.

Introduction

There are 600 species of the genus *Aloe* which belongs to the family of Asphodelaceae [1]. *Aloe* species are furthermore used as purgatives, cosmetics, and skin lotions and are also employed for the treatment of arthritis as well as certain skin irritations, bruises, and burns [1][2][3]. Moreover, the extracts of different *Aloe* species have been reported to be used as anti-aging, anti-inflammatory, anticancer, antibacterial, antifungal, antiviral, antioxidant, and antiseptic treatments [3]. *Aloe vera* (L.) Burm.f. (*Aloe barbadensis* Mill.) is currently being used to treat arthritis, asthma, *Crohn*'s disease, ulcerative colitis, ulcers, acne, psoriasis, eczema, frostbite, burns, cold, and sores [4].

The interesting biological properties described for *A. vera* prompted our group to further investigate its phytochemical composition and biological activity, which resulted in the isolation and characterization of two new *C*-glucosyl chromones named aloeverasides A (**1**) and B (**2**) which were evaluated for their anticancer activity. It was found that compounds **1** and **2** induced a 76.4 and 70.5% growth inhibition, respectively, of the breast cancer cell line (MDA-MB-231) at a concentration of 100 μM. Both compounds were additionally evaluated for their 1,1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant activity, and urease and α-glucosidase enzyme inhibition activities. It was found that compounds **1** and **2** displayed good urease enzyme inhibition activities of 62 and 55%, respectively, and that the antioxidant activity of compound **1** had an inhibition of 60% while that of compound **2** showed potent antioxidant activity with 80% inhibition at 1 mg/ml concentration.

Results and Discussion

A phytochemical investigation of resin of *A. vera* provided two new compounds *viz*., aloeverasides A (**1**) and B (**2**; *Fig. 1*). The molecular formula of aloeveraside A (**1**) was confirmed to be $C_{29}H_{30}O_{11}$ based on the molecular ion peak in the ESI-MS spectrum and 1D- and 2D-NMR spectroscopic analysis.

Moreover, the IR spectrum illustrated the presence of OH (3304 cm^{-1}), ester, and ketone (1705 cm^{-1}) functional groups, as well as aryl rings (1640 cm^{-1}).

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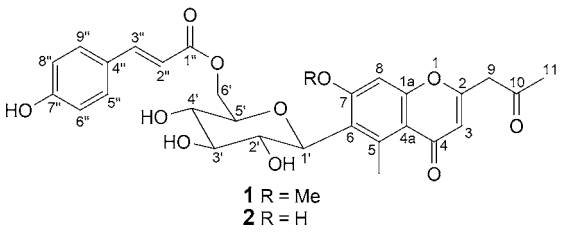

Fig. 1. Structures of aloeverasides A (**1**) and B (**2**).

Preliminary inspection of the ^{1}H-NMR spectrum (see *Experimental Part*) showed the anomeric signal for one *C*-glucosyl group (δ(H) 5.02 (*d*, *J* = 9.6, 1 H, H–(1′), which was further confirmed by the typical *C*-glucosyl signals in the ^{13}C-NMR spectrum (δ(C) 79.9 (C(3′)), 78.5 (C(5′)), 75.6 (C(1′)), 72.3 (C(2′)), 72.1 (C(4′)), and 65.4 (C(6′))) [5][6]. Importantly, the relatively large *J* value of 9.6 Hz for the anomeric H-atom indicated a β-configuration of attachment of the glucose moiety. Furthermore, the NMR spectra of compound **1** illustrated the presence of a *p*-coumaroyl ester (δ(H) 7.66 (*d*, *J* = 16.2, H–C(3″)); δ(C) 146.2; 6.46 (*d*, *J* = 16.2, H–C(2″)); δ(C) 116.2; δ(H) 7.56 (*d*, *J* = 8.4, H–C(5″), H–C(9″)); δ(C) 131.0; δ(H) 6.96 (*d*, *J* = 8.4, H–C(6″), H–C(8″)); δ(C) 115.3) and configuration of C(2″) and C(3″) was *trans* as evidenced from their large coupling constant (*J* = 16.2). Moreover, attachment of *p*-coumaroyl ester was confirmed to be at C(6′) from the clear HMBC between H–C(6′) and C(1″) [5][6].

The ^{1}H-NMR spectrum of compound **1** furthermore illustrated the presence of two aromatic *singlet*s (δ(H) 6.60 (*s*, H–C(8)), 6.09 (*s*, H–C(3))) and one aromatic Me group (δ(H) 2.68 (*s*, Me(5)), while ^{13}C-NMR spectrum illustrated a strong signal for a ketone of a chromone group at δ(C) 181.8. Further NMR analysis of compound **1** showed that it contains an acetonyl group in 2-position, which is evident from the NMR peaks at (δ(H) 3.21 (br. *s*, CH_2(9)); δ(C) 48.5; δ(H) 2.26 (*s*, Me(11)); δ(C) 30.7 and a ketone C=O signal δ(C) 204.6, C(10)) [5][6]. The complete structural elucidation of compound **1** was accomplished by ^{1}H,^{1}H-COSY and HMBC experiments (*Fig. 2*), as well as by comparing NMR chemical shift values with other *C*-glucosyl chromones [5][6]. Furthermore, the key HMBCs between H–C(8) and C(1a), C(4a), C(6), and C(7); H–C(9) and C(2), C(3), C(10), and C(11); H–C(11) and C(9), C(10); Me(5) and C(4a), C(5), and C(6); MeO and C(7); H–C(1′) and C(3′), C(5), C(5′), and C(7); H–C(3) and C(2), C(4), C(4a), and C(9) confirmed the 6′-*O*-coumaroyl-*C*-glucosyl chromone skeleton as well as the substitution and linkages of compound **1** [5][6]. Therefore, compound **1** was identified as 6′-*O*-coumaroyl 2-acetonyl-6-β-D-glucopyranosyl-7-methoxy-5-methylchromone.

The molecular formula of aloeveraside B (**2**) was determined to be $C_{28}H_{28}O_{11}$ on the basis of ESI-MS and NMR data. The structure of **2** was determined by comparison of its NMR data with those of aloeveraside A (**1**). The major difference observed in the spectra of compound **2** and **1** is that the MeO signal present in the latter, *viz.*, (δ(H) 3.83 (*s*, MeO), δ(C) 55.8 (MeO)), was absent in the NMR spectra of **2**. This is further supported by the mass spectrum, which showed a molecular ion of 14 amu less than for compound **1**. This significant fact that the MeO group at C(7) is absent in compound **2** and replaced by an OH together with the otherwise striking similarities between the rest of the spectra allowed for the structural assignment of aloeveraside B (**2**) as 6′-*O*-coumaroyl 2-acetonyl-6-β-D-glucopyranosyl-7-hydroxy-5-methylchromone.

Aloeverasides A (**1**) and B (**2**) were tested for their antiproliferative potential against cancer cells in culture (*Fig. 3*). Breast cancer cells MDA-MB-231 were treated with four different concentrations (25, 50, 75, and 100 μM) of the two compounds, and an MTT assay for cell viability was subsequently performed. The results illustrated in *Fig. 3* indicate that both the compounds were able to induce a concentration-dependent decrease in the viability of the cancer cells. However, for compound **2** this effect appears pronounced only at a concentration of 50 μM and higher. Interestingly, at each concentration tested, compound **1** exhibits a greater degree of cell growth inhibition as compared to compound **2**, except at 75 μM, where both compounds show comparable cytotoxicity (about 46 – 48%). It is noteworthy that considerable cell growth

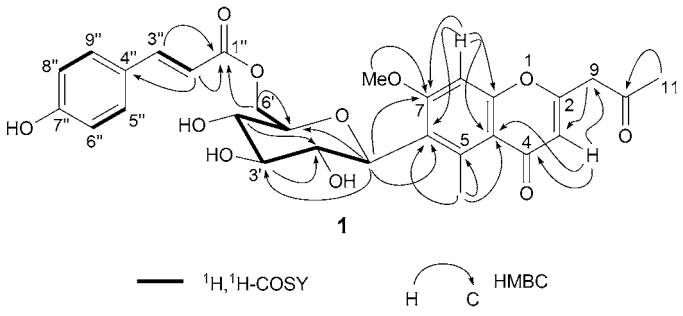

Fig. 2. Key COSY correlations and HMBCs of aloeveraside A (**1**).

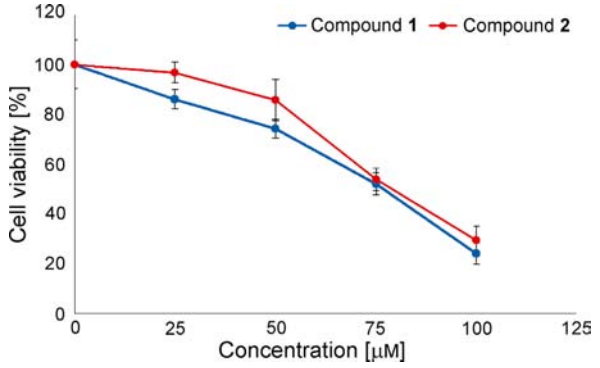

Fig. 3. Effect of compounds **1** and **2** on the proliferation of breast cancer cells in culture. MDA-MB-231 breast cancer cells (1×10^4) were treated for 24 h with varying concentrations of the compounds **1** and **2** as shown above in the figure. The effects of the two compounds on cell proliferation were detected by the MTT assay as described in '*Experimental Part*' section. All results are expressed as percentage of control ± SD of quadruplicate determinations.

inhibition of around 76 and 70.5% is observed at the higher concentration of 100 μM for compounds **1** and **2**, respectively. Therefore, the present results suggest that although both novel compounds **1** and **2** were capable of inducing cytotoxic effects against breast cancer cells in a concentration-dependent manner, the former was slightly more potent.

Experimental Part

General

For TLC, precoated Al sheets (SiO_2 *60F-254*, *E. Merck*) were used. Visualizations of the TLC plates were achieved under the UV light at 254 and 366 nm and also by spraying with the $Ce(SO_4)_2$ reagent followed by heating. Optical rotations were measured on a *KRUSS P P3000* polarimeter (*A. Kruss Optronic*, Germany). IR Spectra were recorded on a *Bruker*, *ATR-Tensor 37* spectrophotometer. Multinuclear and multidimensional NMR spectra were recorded on a BRUKER NMR spectrometer operating at 600 MHz (150 MHz for ^{13}C) with cryoprobe prodigy. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are given in Hz. ESI-MS spectra were recorded on a *Waters Quattro Premier XE* Mass Spectrometer (*Waters*, Milford, MA).

Sample Collection and Identification

The resin of *A. vera* was purchased from a local market in Nizwa, Sultanate of Oman and identified by Mr. *Saif Al-Hatmi* (plant taxonomist) at the Oman Botanical Garden Muscat, Oman. A voucher specimen (No. AFS-08/2016) was deposited with the Oman Botanical Garden Muscat.

Extraction and Purification

The air-dried powdered resin (1 kg) of *A. vera* was finely extracted with MeOH (5 l) at r.t. (3 × 15 days) and evaporated under reduced pressure to yield a crude MeOH extract (987 g). The resulting crude MeOH extract was successively fractionated into hexane (0.9 g), CH_2Cl_2 (8.5 g), AcOEt (73.2 g), BuOH (602.0 g), and aqueous (280.0 g). A TLC inspection of the CH_2Cl_2 and AcOEt extracts exhibited such a strong similarity in their nature that they were combined for chromatographic purification (81.7 g). Thus, CC (70 – 230 mesh; *Merck*), using hexane, hexane/AcOEt, AcOEt, AcOEt/MeOH, and pure MeOH with 10% increasing polarity to afford 28 fractions ($F_1 - F_{28}$). *Frs.* F_{18-21} were combined due to their similar TLC profiles (250 mg) and subjected to a further CC using hexane/AcOEt with increasing polarity (2:8, 4:6, 6:4, and 8:2) to afford six subfractions (F_{A-F}). Of these subfractions, F_B was further subjected to CC to afford the two new aloeverasides *viz*., compound **1** (15 mg; R_f ($MeOH/CH_2Cl_2$ (2:8)) 0.7) and compound **2** (20 mg; R_f ($MeOH/CH_2Cl_2$ (2:8) 0.5)) eluting with 2% MeOH/AcOEt.

Aloeveraside A (= **(1*S*)-1,5-Anhydro-6-*O*-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-1-[7-methoxy-5-methyl-4-oxo-2-(2-oxopropyl)-4*H*-chromen-6-yl]-D-glucitol**; **1**). Yellow solid. UV (CH_2Cl_2): 253 (3.96), 276 (3.71), 330 (3.58). IR (KBr): 3304, 1705, 1640, 1370, 1080. ^{1}H-NMR (600 MHz, CD_3OD): 7.66 (*d*, *J* = 16.2, H–C(3″)); 7.56 (*d*, *J* = 8.4, H–C(5″), H–C(9″)); 6.96 (*d*, *J* = 8.4, H–C(6″), H–C(8″)); 6.60 (*s*, H–C(8)); 6.46 (*d*, *J* = 16.2, H–C(2″)); 6.09 (*s*, H–C(3)); 5.02 (*d*, *J* = 9.6, H–C(1′)); 4.57 (*dd*, *J* = 1.8, 12.0, H_a–C(6′)); 4.34 (*dd*, *J* = 6.6, 12.0, H_b–C(6′)); 3.83 (*s*, MeO); 3.66 –3.70 (*m*, H–C(3′)); 3.62 – 3.66 (*m*, H–C(4′)); 3.48 – 3.54 (*m*, H–C(2′), H–C(5′)); 3.21 (br. *s*, CH_2(9)); 2.68 (*s*, Me(5)); 2.26 (*s*, Me(11)). ^{13}C-NMR (150 MHz, CD_3OD): 204.6 (C(10)); 181.8 (C(4)); 169.1 (C(1″)); 163.1 (C(2)); 161.1 (C(7)); 160.4 (C(7″)); 157.9 (C(1a)); 146.2 (C(3″)); 146.1 (C(5)); 131.0 (C(5″), C(9″)); 128.3 (C(6), C(4″)); 121.1 (C(8)); 116.0 (C(2″)); 115.3 (C(6″), C(8″)); 114.3 (C(4a)); 111.0 (C(3)); 79.9 (C(3′)); 78.5 (C(5′)); 75.6 (C(1′)); 72.3 (C(2′)); 72.1 (C(4′)); 65.4 (C(6′)), 55.8 (MeO), 48.5 (C(9)); 30.7 (C(11)); 23.3 (Me(5)). ESI-MS: 577 (70, $[M + Na]^+$).

Aloeveraside B (= **(1*S*)-1,5-Anhydro-1-[7-hydroxy-5-methyl-4-oxo-2-(2-oxopropyl)-4*H*-chromen-6-yl]-6-*O*-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-D-glucitol**; **2**). Yellow solid. UV (CH_2Cl_2): 255 (3.90), 271 (3.70), 330 (3.58). IR (KBr): 3280, 1705, 1645, 1450, 1370, 1250, 1020. ^{1}H-NMR (600 MHz, CD_3OD): 7.63 (*d*, *J* = 16.2, H–C(3″)); 7.45 (*d*, *J* = 8.4, H–C(5″), H–C(9″)); 6.80 (*d*, *J* = 8.4, H–C(6″), H–C(8″)); 6.69 (*s*, H–C(8)); 6.39 (*d*, *J* = 16.2, H–C(2″)); 6.14 (*s*, H–C(3)); 5.02 (*d*, *J* = 9.6, H–C(1′)); 4.57 (*dd*, *J* = 1.8, 12.0, H_a–C(6′)); 4.32 – 3.36 (*m*, H_b–C(6′)); 4.06 – 4.10 (*m*, H–C(4′)); 3.66 – 3.70 (*m*, H–C(3′)); 3.48 – 3.54 (*m*, H–C(2′), H–C(5′)); 3.21 (br. *s*, CH_2(9)); 2.68 (*s*, Me(5)); 2.26 (*s*, Me(11)). ^{13}C-NMR (150 MHz, CD_3OD): 204.4 (C(10)); 181.9 (C(4)); 169.2 (C(1″)), 162.3 (C(2)); 161.3 (C(1a), C

(7″)); 160.1 (C(7)); 146.2 (C(3″)); 143.1 (C(5)); 131.2 (C(5″), C(9″)); 133.6 (C(4″)); 127.0 (C(6)); 119.4 (C(8)); 114.9 (C(2″)); 116.8 (C(6″), C(8″)); 115.8 (C(4a)); 113.2 (C(3)); 80.0 (C(3′)); 79.8 (C(5′)); 75.5 (C(1′)); 72.9 (C(4′)); 71.9 (C(2′)); 65.2 (C(6′)), 48.7 (C(9)); 29.8 (C(11)); 23.3 (Me(5)). ESI-MS: 563 (94, $[M + Na]^+$).

Cell Line and Reagents. The breast cancer cell line MDA-MB-231 was maintained in DMEM (*Invitrogen*, Carlsbad, CA, USA) media. The media was supplemented with 10% fetal bovine serum and 1% antimycotic antibiotic (*Invitrogen*, Carlsbad, CA, USA). Cells were cultured in a 5% CO_2 – humidified atmosphere at 37 °C. A 5 mg/ml stock soln. of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared in PBS.

MTT Assay for Assessing Cell Viability. Cells were seeded at a density of 1×10^4 cells per well in 96-well microtiter culture plates. After overnight incubation, normal growth medium was removed and replaced with either fresh medium (untreated control) or different concentrations of respective compounds in growth medium diluted from a 2 mM stock. After 24 h of incubation, MTT soln. was added to each well (0.1 mg/ml in DMEM) and incubated further for 4 h at 37 °C. Upon termination, the supernate was aspirated and the MTT formazan, formed by metabolically viable cells, was dissolved in a solubilization soln. containing DMSO (100 µl) by mixing for 5 min on a gyratory shaker. The absorbance was measured at 540 nm (reference wavelength 690 nm) on an *Ultra Multifunctional Microplate Reader* (*Bio-Rad*, USA). Absorbance of control (without treatment) was considered as 100% cell survival. Each treatment had four replicate wells.

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