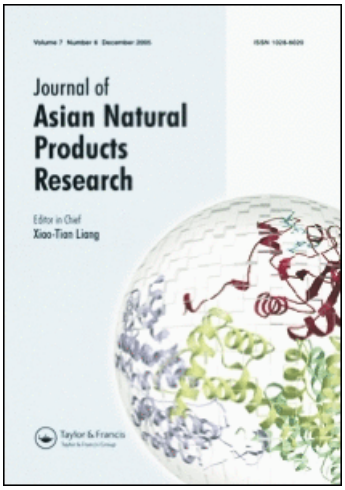

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ORIGINAL ARTICLE

Three new antioxidant *C*-glucosylanthrones from *Aloe nobilis*

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Three new *C*-glucosylanthrones, 3′-*O*-acetyl-5-hydroxylaloin A (**2**), 2′,6′-*O*-diacetyl-5-hydroxylaloin A (**4**), and 4′,6′-*O*-diacetyl-5-hydroxylaloin A (**5**), along with three known compounds, 5-hydroxyaloin A (**1**), 6′-acetyl-5-hydroxylaloin A (**3**), and 4-methoxy-6-(2′,4′-dihydroxy-6′-methylphenyl)-pyran-2-one (**6**), were isolated from the leaves of *Aloe nobilis*, and their structures were elucidated on the basis of spectroscopic evidences. Compounds **1**, **2**, **4** and **5** showed antioxidant activity with inhibitory rates of 31.0, 34.0, 34.0, and 42.0%, respectively, at 10^{-5} M.

Keywords: *Aloe nobilis*; Liliaceae; *C*-glucosylanthrones; aloin A; antioxidant activity

1. Introduction

Aloe sp. have been widely used as folk medicines for thousands of years. The composition of *Aloe* leaf exudates can generally be classified into two main groups, namely chromones and anthraquinones [1]. As a part of systematic chemical studies on *Aloe nobilis* [2], we have investigated the constituents of its leaf exudates. Six compounds (**1**–**6**) were isolated, three of which were characterized as new anthraquinones, i.e. 3′-*O*-acetyl-5-hydroxylaloin A (**2**), 2′,6′-*O*-diacetyl-5-hydroxylaloin A (**4**), and 4′,6′-*O*-diacetyl-5-hydroxylaloin A (**5**) (Figure 1). Their antioxidant activities were also evaluated.

2. Results and discussion

From the MeOH extract of *A. nobilis*, three new *C*-glucosylanthrones, **2**, **4**, and **5**, along with three known compounds, 5-hydroxyaloin A (**1**) [3], 6′-acetyl-5-hydroxylaloin A (**3**) [4], and 4-methoxy-6-(2′,4′-dihydroxy-6′-methylphenyl)-pyran-2-one (**6**) [5], were isolated.

3′-*O*-acetyl-5-hydroxylaloin A (**2**) was obtained as a yellow amorphous powder. The positive-ion ESI-MS showed the pseudomolecular ion at *m/z* 477.2 $[M + H]^+$ and 499.2 $[M + Na]^+$, suggesting the molecular formula $C_{23}H_{24}O_{11}$, which was confirmed by the HR-ESI-MS. The ^{1}H NMR spectral data (Table 1) of **2** showed characteristic signals of 5-hydroxylaloin A as oxymethylene proton H-11 (δ 4.59, s), a methine proton H-10 (δ 4.78, d, $J = 2.0$ Hz), two *ortho*-coupled aromatic protons H-6 (δ 6.70, d, $J = 8.8$ Hz) and H-7 (δ 7.02, d, $J = 8.8$ Hz), as well as two *meta*-coupled aromatic protons H-2 (δ 6.84, br s) and

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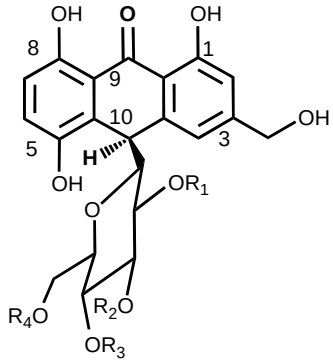

	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	Ac	H	H
3	H	H	H	Ac
4	Ac	H	H	Ac
5	H	H	Ac	Ac

Figure 1. Structures of compounds **1**–**5**.

H-4 (δ 7.12, br s). Signals at 1.95 ppm in the ^{1}H NMR spectrum, and 21.0 and 172.8 ppm in the ^{13}C NMR spectrum indicated the presence of an acetyl group. The downfield shift of the signal corresponding to the C-3′ (from δ 79.2 in **1** to δ 80.5 in **2**) and the upfield shift of the signals corresponding to the C-2′ (from δ 73.2 in **1** to δ 71.5 in **2**) and C-4′ (from δ 72.0 in **1** to δ 69.9 in **2**) were in agreement with the esterification of the 3′-hydroxyl group, thus placing the acetate unit at C-3′ of the glucose moiety.

Rauwald and Beil [3] have demonstrated that the glucose moiety at C-10 in 5-hydroxylaloin A (**1**) only naturally existed in β-orientation, in contrast to that in aloin existing as a mixture of α- and β-orientation. All of the above information was in accord with 3′-*O*-acetyl-5-hydroxylaloin A as the structure of compound **2**.

2′,6′-*O*-diacetyl-5-hydroxylaloin A (**4**) was obtained as a yellow amorphous powder. The positive HR-ESI-MS showed an $[M + H]^+$ ion at *m/z* 519.1522, suggesting the molecular formula as $C_{25}H_{26}O_{12}$. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **4** were similar to those of **1** except for the signals of two additional acetyl moieties in **4**. The positions for these two acetyl groups were assigned by HMBC correlations, i.e. H-2′ (δ 4.09) to the acetyl carbonyl group at δ 171.8 and H-6′ (δ 4.30, 4.12) to the acetyl carbonyl group at δ 172.9. Other key correlations of H-2 at δ 6.86 and H-4 at δ 7.12 to C-11 at δ 64.0, H-10 to C-1′ at δ 80.7, H-1′ at δ 3.98 to C-4a at δ 143.8 and C-5a at δ 127.0 further proved the skeleton of 5-hydroxylaloin A in **4**. All the above information supported the structure of **4** as 2′,6′-diacetyl-5-hydroxylaloin A.

4′,6′-*O*-diacetyl-5-hydroxylaloin A (**5**) was obtained as a yellow amorphous powder. The positive ESI-MS showed an $[M + H]^+$ ion at *m/z* 519.2 and $[M + Na]^+$ ion at *m/z* 541.2. The HR-positive ESI-MS spectrum showed an $[M + H]^+$ ion at *m/z* 519.1550, suggesting the molecular formula $C_{25}H_{26}O_{12}$. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **5** demonstrated that **5** is an isomer of **4**, and the difference in their structures is the linked position of two acetyl groups. Furthermore, the linked positions of these two acetyl groups were determined by HMBC correlations, i.e. H-4′ at δ 4.33 to the acetyl carbonyl at δ 171.9 and H-6′ at δ 3.74 to the acetyl carbonyl at δ 172.5. All these results indicated that the structure of **5** was 4′,6′-*O*-diacetyl-5-hydroxylaloin A.

In addition, compounds **1**, **2**, **4** and **5** showed strong antioxidant activity in the microsomal lipid peroxidation induced by ferrous-cysteine assay. The inhibition rates of malondialdehyde (MDA) for compounds **1**, **2**, **4** and **5**, at a concentration of 1.0×10^{-5} M, were 31.0, 34.0, 34.0 and

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **2**, **4** and **5**[a] (δ in CD_3OD).

	2		**4**		**5**	
No	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	163.3		163.7		162.9	
2	114.3	6.84 br s	114.5	6.86 s	114.2	6.82 s
3	152.0		156.8		152.3	
4	118.8	7.12 br s	119.6	7.12 s	117.8	7.07 s
5	147.3		147.7		147.4	
6	118.5	6.70 d ($J = 8.8$)	125.2	6.71 d ($J = 8.8$)	124.8	6.70 d ($J = 8.8$)
7	124.9	7.02 d ($J = 8.8$)	117.4	7.03 d ($J = 8.8$)	117.7	7.02 d ($J = 8.8$)
8	156.9		156.8		156.9	
9	195.6		195.3		195.5	
10	41.9	4.78 d ($J = 2.4$)	42.0	4.74 br s	40.6	4.79 br s
11	64.5	4.59 s	64.0	4.60 s	64.5	4.58 s
1a	117.3		117.2		118.0	
4a	145.0		143.8		146.0	
5a	127.6		127.0		126.3	
8a	118.5		117.7		119.1	
1′	84.2	3.66 dd ($J = 2.4$, 9.6)	80.7	3.98 dd ($J = 2$, 10.0)	85.6	3.38*
2′	71.5	3.08 m	73.6	4.09 dd ($J = 9.6$, 8.8)	73.0	2.97 t ($J = 9.6$)
3′	81.5	3.49 m	78.6	3.21 t ($J = 9.2$, 8.8)	76.3	3.19*
4′	69.9	2.80 t ($J = 9.6$, 9.6)	71.2	3.03 t ($J = 9.2$, 9.6)	72.3	4.33 t ($J = 9.6$, 9.2)
5′	80.5	3.08 m	79.0	3.30 m	76.6	3.34 m
6′	62.9	3.52 m 6a, 3.68 m 6b	64.9	4.30 dd ($J = 2$, 12) 4.12 m	64.0	3.74 m
2′-CH_3CO			171.8			
2′-CH_3CO			20.8	2.04 s		
3′-CH_3CO	172.8					
3′-CH_3CO	21.0	1.95				
4′-CH_3CO					171.9	
4′-CH_3CO					20.7	1.93 s
6′-CH_3CO			172.9		172.5	
6′-CH_3CO			21.0	2.01 s	20.5	1.91 s

Notes: [a] Measured in CD_3OH at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, respectively, with assignments confirmed by HMQC and HMBC. δ in ppm and J in Hz.

42.0%, respectively, compared with 21.9% for vitamin E, a well-known antioxidant.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a PE-241 digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer. IR spectra were run on a Nicolet Impact-400 spectrometer. NMR spectra were recorded on a Varian MERCURY-400 and INOVA-500 spectrometer using TMS as an internal standard, and chemical shifts are given in ppm. ESI-MS measurements were carried out on an Aglient 1100 series LC/MSD Trap SL mass spectrometer. HR-ESI-MS were obtained from an Agilent 1100 series LC/MSD TOF. Silica gel (100–200 mesh; Qingdao Marine Chemical Company, Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and RP-18 (40–70 μm; YMC Co. Ltd, Tokyo, Japan) were used for column chromatography.

3.2 Plant material

A. nobilis was collected from the *Aloe* cultivated base of Ruiwangfen, Beijing, China, in 2004, and identified by Prof. Lin Ma. The authenticated sample (No. 21572) of the plant is deposited at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

Dried leaves (5 kg) of *A. nobilis* were segmented and soaked in MeOH at room temperature for 1 week. The resulting extract was filtered and concentrated to dryness under reduced pressure to furnish a brown residue (210 g), which was suspended in water and partitioned with petroleum ether, EtOAc and *n*-BuOH successively. The EtOAc-soluble residue (13.0 g) was subjected to column chromatography over silica gel, eluting with $CHCl_3/CH_3OH$ gradiently to afford 11 fractions I–XI. Fraction IV (1.5 g) was chromatographed over a Sephadex LH-20 column eluting with 50% MeOH/H_2O to give five fractions (Frs IV-a–IV-e). Fraction IV-b was chromatographed over a Sephadex LH-20 column eluting with 50% MeOH/H_2O followed by preparative TLC (CH_2Cl_2:MeOH:H_2O = 80:20:1) to provide compounds **1** (120 mg, R_f = 0.30), **2** (25 mg, R_f = 0.35), and **3** (180 mg, R_f = 0.39). Fraction IV-d was subjected to silica gel column chromatography eluting with 5% MeOH in CH_2Cl_2 to give compounds **4** (130 mg) and **5** (140 mg). Fraction VII was rechromatographed over a Sephadex LH-20 column eluting with MeOH followed by preparative TLC (CH_2Cl_2:MeOH = 50:1, R_f = 0.45) to give compound **6** (20 mg).

3.3.1 3′-O-acetyl-5-hydroxylaloin A (2)

Yellow amorphous powder (25 mg), $[\alpha]_D^{20}$ + 6.0 (c = 0.10, MeOH). UV λ_{max}: 204 nm; IR ν_{max} (KBr): 3378.7, 1726.6, 1639.2, 1621.8, 1581.6, 1464.7, 1374.3, 1271.2, 1231.2, 1046.2 cm^{-1}. ^1H and ^{13}C NMR spectral data see Table 1. ESI-MS: *m/z* 477.2 $[M + H]^+$ and 499.2 $[M + Na]^+$. HR-ESI-MS: *m/z* 499.1222 (calcd for $C_{23}H_{24}O_{11}Na$, 499.1216).

3.3.2 2′,6′-O-diacetyl-5-hydroxylaloin A (4)

Yellow amorphous powder (130 mg), $[\alpha]_D^{20}$ + 8.0 (c = 0.10, MeOH). UV λ_{max}: 204 nm; IR ν_{max} (KBr): 3390.2, 1719.1, 1639.2, 1622.1, 1581.8, 1464.8, 1373.2, 1273.3, 1230.2, 1049.1 cm^{-1}. ^1H and ^{13}C NMR spectral data see Table 1. HR-ESI-MS: *m/z* 519.1522 $[M + H]^+$ (calcd for $C_{25}H_{27}O_{12}$, 519.1503).

3.3.3 4′,6′-O-diacetyl-5-hydroxylaloin A (5)

Yellow amorphous powder (140 mg), $[\alpha]_D^{20}$ + 1.7 (c = 0.24, MeOH). UV λ_{max}:

203 nm; IR ν_{max} (KBr): 3333.5, 1724.3, 1621.6, 1581.3, 1464.3, 1374.2, 1275.3, 1235.0, 1043.6 cm^{-1}. ^1H and ^{13}C NMR spectral data see Table 1. HR-ESI-MS: *m/z* 519.1550 $[M + H]^+$ (calcd for $C_{25}H_{27}O_{12}$, 519.1503).

3.4 Antioxidant activity assay

According to the reported procedures [6], the antioxidant activities of compounds **1**–**6** were determined by measuring the content of MDA, a compound that is produced during microsomal lipid peroxidation induced by ferrous-cysteine. MDA was detected by using the thiobarbituric acid (TBA) method. Briefly, 1 mg/ml microsomal protein, different concentrations of the compound or the vehicle, and 0.2 mM cysteine in 0.1 M PBS were incubated for 15 min at 37°C. After the addition of 0.5 mM ferrous sulfate, the mixture was incubated again for 15 min at 37°C. An equal volume of 20% trichloroacetic acid was added to terminate the reaction, and the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was reacted with 0.67% TBA for 10 min at 100°C. After cooling, the DNA was quantified by determining the absorbance at 532 nm and then the inhibitory rate (IR) was calculated.

The IR was calculated as $IR(5) = 100\% - At/(Ap - Ac) \times 100\%$, where Ap, At and Ac refer to the absorbance of ferrous-cysteine, test compound, and solvent control groups, respectively.

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