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Antiviral anthraquinones from the roots of *Knoxia valerianoides*

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ABSTRACT

Three new anthraquinones, (2*S*)-8-carboxy-9-hydroxy-2-(2-hydroxypropan-2-yl)-1,2-dihydroanthra[2,1-b]furan-6,11-dione (**1**), 1,2,3,6-tetrahydroxy-9,10-anthraquinone (**2**), and 1,2,3,5,6-pentahydroxy-9,10-anthraquinone (**3**), as well as four known 9,10-anthraquinones (**4**–**7**) and five known triterpenes (**8**–**12**), were isolated from the roots of *Knoxia valerianoides*. Their structures and the absolute configuration of **1** were determined through interpretation of spectroscopic data, including UV, IR, NMR and CD spectra. The isolates were evaluated for their antiviral activities, and compounds **1** and **4** showed inhibitory effects on Coxsackie virus B3 replication with IC_{50} values of 19.24 μM and 11.11 μM, respectively. Compound **4** showed activity against influenza virus A/Hanfang/359/95 with an IC_{50} value of 11.11 μM.

1. Introduction

The genus *Knoxia* consists of approximately nine species distributed in the tropical areas of Asia and Oceania, but only three species belonging to this genus, namely, *Knoxia valerianoides*, *Knoxia corymbosa* and *Knoxia mollis*, are found in China, particularly in the southern regions. The roots of *K. valerianoides* are used as a traditional Chinese medicine and have long been used as purgative and anti-ulcer agents. Only a few anthraquinones have been obtained from *K. valerianoides*, and some of these anthraquinones have been found to exhibit inhibitory activities against rat lens aldose reductase and the formation of advanced glycation end products (Wang et al., 1985; Yuan and Zhao, 2005, 2006; Yoo et al., 2010; Zhou et al., 1994).

As part of our search for bioactive compounds from Chinese medicinal plants, our group has investigated *K. valerianoides*. In our previous study, 34 9,10-anthraquinones and other classes of secondary metabolites, including triterpenes, steroids, lignanoids and coumarin, were isolated and identified from several fractions of its ethanol extract, and some of these compounds showed anti-inflammatory and hepatoprotective activities (Zhao et al., 2011a,b; Zhao et al., 2012a,b). Further investigation of the remaining fractions of the same extract afforded three new anthraquinones (**1**–**3**) and nine known compounds (**4**–**12**) (Fig. 1), and their antiviral activities against Coxsackie virus B3 and influenza virus A/Hanfang/359/95 were evaluated through the cytopathic effect (CPE) inhibition assay.

2. Results and discussion

Compound **1** was isolated as a yellowish, amorphous solid. Its molecular formula was determined as $C_{20}H_{16}O_7$ from its HRESIMS (367.0820 $[M-H]^-$, calcd. for $C_{20}H_{15}O_7$, 367.0823) and ^{13}C NMR data and indicated thirteen degrees of unsaturation. The IR spectrum displayed major absorption bands at 3406 cm^{-1} (hydroxy group), 1670 cm^{-1} (conjugated carbonyl), 1581 and 1462 cm^{-1} (aromatic ring). The ^1H NMR spectrum of **1** showed resonances for two *ortho*-coupled aromatic protons at δ_H 7.20 (1H, d, J = 8.4 Hz, H-7) and 8.02 (1H, d, J = 8.4 Hz, H-8), two isolated aromatic protons at δ_H 7.47 (1H, s, H-4) and 8.49 (1H, s, H-1); this information in combination with the ^{13}C NMR signals (Table 1) for two carbonyl carbons (δ_C 179.9 and 183.5) and twelve aromatic carbons (in the range δ_C 166.1–114.5) suggested that **1**

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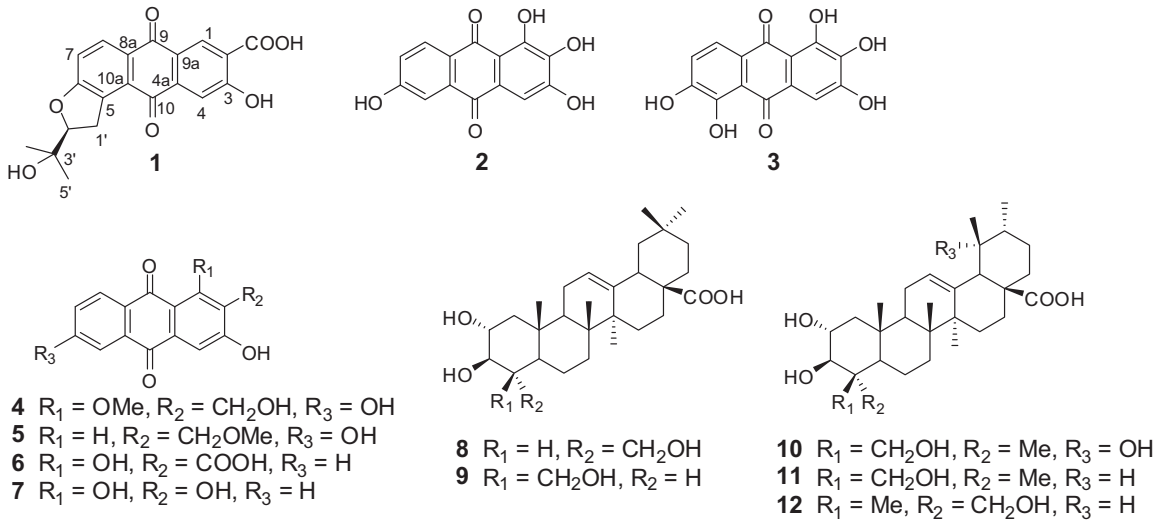

Fig. 1. Structures of compounds **1**–**12**.

was a tetra-substituted 9,10-anthraquinone derivative (El-Gamal et al., 1995; Ling et al., 2002). Using the HSQC data, the remaining signals in the NMR spectrum of **1** were attributed to a methylene [δ_H 3.56 (2H, d, J = 8.6 Hz), δ_C 31.7, CH_2-1′], an oxymethine [δ_H 4.79 (1H, t, J = 8.6 Hz), δ_C 91.8, CH-2′], an oxygenated quaternary carbon (δ_C 70.5, C-3′) and two aliphatic methyl singlets [δ_H 1.18, 1.19 (each 3H, s), CH_3-4′,5′], supporting the presence of a 2′,3′-oxygen-bearing isopent-1′-yl moiety, which was confirmed by the HMBC correlations from H-1′ to C-2′ and C-3′, from H-2′ to C-4′ and C-5′, and from methyl protons to C-2′ and C-3′. In the HMBC spectrum of **1** (Fig. 2), correlations from H-1 to C-2, C-3, C-9 and C-4a, from H-4 to C-2, C-10, C-4a and C-9a, from H-7 to C-5, C-6 and C-8a, and from H-8 to C-6, C-9 and C-10a, together with their chemical shifts, suggested that **1** was a 2,3,5,6-tetra-substituted 9,10-anthraquinone with oxygenated C-3 and C-6. The downfield-shifted CH-2′ (δ_C 91.8) signal and the HMBC correlations from H-2′ to C-5 and C-6 and from H-1′ to C-6 and C-10a indicated an oxygen bridge between C-2′ and C-6, a connection between C-1′ and C-5, and that C-3′ bears an OH group. The remaining one degree of unsaturation accounted for a carboxyl group (δ_C 170.5) located at C-2, as determined from the HMBC correlation of H-1 to the carbonyl carbon of COOH. Consequently, C-3 bears an OH group according to the molecular formula. The optical rotation of **1** was positive ($[\alpha]_D^{20}$ + 68, MeOH), and its CD spectrum exhibited a positive Cotton effect at 295 nm. These data were similar to those obtained for (2′*S*)-dihydrofurano-anthraquinone originally obtained from *K. valerianoides* (Zhao et al., 2011a,b) but opposite to the values published for compounds with a similar chiral center to **1**, such as *R*-(−)-3′,4′-deoxypsorospermin derivatives and *R*-(−)-tubaic acid (Habib et al., 1987; Meepagala et al., 2005), indicating that the C-2′ position is *S*. Therefore, **1** was established as (2*S*)-8-carboxy-9-hydroxy-2-(2-hydroxypropan-2-yl)-1,2-dihydroanthra[2,1-b]furan-6,11-dione.

Table 1
^{1}H and ^{13}C NMR data of compounds **1**–**3**.

No.	**1**[a]		**2**[b]		**3**[b]	
	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1	8.49 (1H, s)	130.9		151.7		152.0
2		124.9		139.1		139.8
3		165.1		151.3		151.2
4	7.47 (1H, s)	114.8	7.22 (1H, s)	108.6	7.28 (1H, s)	108.6
4a		138.4		124.7		124.1
5		130.7	7.44 (1H, d, 2.4)	112.5		151.1
6		166.1		163.3		153.0
7	7.20 (1H, d, 8.4)	114.5	7.19 (1H, dd, 9.0, 1.8)	120.8	7.63 (1H, d, 8.4)	120.2
8	8.02 (1H, d, 8.4)	129.8	8.06 (1H, d, 8.4)	129.4	7.17 (1H, d, 8.4)	120.8
8a		126.8		124.8		123.5
9		179.9		186.4		185.7
9a		119.7		110.0		110.4
10		183.5		181.1		187.4
10a		130.3		135.6		116.1
2-COOH		170.5				
1-OH			12.95 (1H, s)		12.90 (1H, s)	
2-OH			9.86 (1H, brs)		10.04 (1H, brs)	
3-OH			10.67 (1H, brs)		10.76 (1H, brs)	
5-OH					13.06 (1H, s)	
6-OH			11.04 (1H, brs)		10.88 (1H, brs)	
1′	3.56 (2H, d, 8.6)	31.7				
2′	4.79 (1H, t, 8.6)	91.8				
3′		70.5				
4′	1.19 (3H, s)	25.5				
5′	1.18 (3H, s)	26.3				

[a] Data were measured in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C.
[b] Data were measured in DMSO-d_6 at 600 MHz for ^{1}H and 150 MHz for ^{13}C.

Compounds **2** and **3** were obtained as a mixture of an orange, amorphous solid at a ratio of 4:1, as evidenced by the integral area of the proton signal. Compound **2** had the molecular formula of $C_{14}H_8O_6$, as assigned by HRESIMS at m/z 271.0243 $[M-H]^-$. The UV and IR spectrum showed typical absorptions for 9,10-anthraquinone. The ^{1}H NMR spectrum of **2** showed resonances assignable to an isolated aromatic proton (δ_H 7.22, 1H, s), a set of ABX-coupled protons [δ_H 7.44 (1H, d, J = 2.4 Hz), 7.19 (1H, dd, J = 9.0, 1.8 Hz) and 8.06 (1H, d, J = 8.4 Hz)], and four phenolic hydroxy protons, one of which was hydrogen-bonded (δ_H 12.95, 1H, s) (Table 1). These data suggested that **2** was a tetrahydoxy-9,10-anthraquinone. The resonances of proton and protonated carbon in the NMR spectrum of **2** were assigned unambiguously based on the HSQC experiment. The substitution pattern was determined by the HMBC correlations (Fig. 2) from H-4 to C-2, C-3, C-10 and C-9a, from H-5 to C-7,