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New anthraquinone dimer from the root bark of *Cassia artemisioides* (Gaudich. Ex. DC) Randell

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New anthraquinone dimer from the root bark of *Cassia artemisioides* (Gaudich. Ex. DC) Randell

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The phytochemical investigation of the root bark of *Cassia artemisioides* (Gaudich. Ex. DC) Randell resulted in the isolation of one new anthraquinone 1,1′-dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-*O*-bianthraquinone (**1**) along with four known anthraquinones 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (**2**), 1-hydroxy-8-methoxy-3-methylanthraquinone (**3**), 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (**4**), and 1,6,8-trihydroxy-3-methylanthraquinone (**5**). The structures of the compounds were elucidated using spectroscopic techniques including 1D and 2D NMR. The compounds were evaluated for antioxidant activity. 1,6,8-Trihydroxy-3-methyl anthraquinone (**5**) showed good activity among the tested compounds.

Keywords: Leguminosea; *Cassia artemisioides* (Gaudich. Ex. DC) Randell; anthraquinones; antioxidant

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1. Introduction

Cassia species are a rich source of flavonoids [1] and anthraquinones [2] and several of them have medicinal values [3] such as hypoglycemic [4], hepatoprotective [5], hypocholesterolemic [6], antibacterial [7], and antidiabetic [8] properties. In Ayurvedic medicine, *Cassia* roots are used for the treatment of skin diseases, leprosy, tuberculous glands, and syphilis, while its fruits are used for the treatment of inflammation, throat troubles, liver complaints, chest complaints, rheumatism, and asthma [9].

Several antioxidants of plants' origin are widely used against oxidative stress [10] and several *Cassia* species are reported to have antioxidant activities [11]. As lipid peroxidation causes damage to the cell membrane, leading to liver injury, atherosclerosis, kidney damage, aging, and susceptibility to cancer [12], antioxidants may play a role in preventing some of these conditions [13]. A close relationship between antioxidant activity and antimutagenicity has been demonstrated by Yen and Chen [14]. The roasted jue ming zi used as a health drink tea is reported to be antimutagenic [15].

Polyphenols are known as antioxidant agents, and the presence of a carbonyl group seems to enhance their activities. For example, emodin with three hydroxyl and two carbonyl groups exhibits antioxidant properties [16].

In the present investigation, we report the isolation, structural elucidation, and evaluation of the antioxidant activities of one new and four known anthraquinones **1**–**5** (Figure 1) isolated from the root barks

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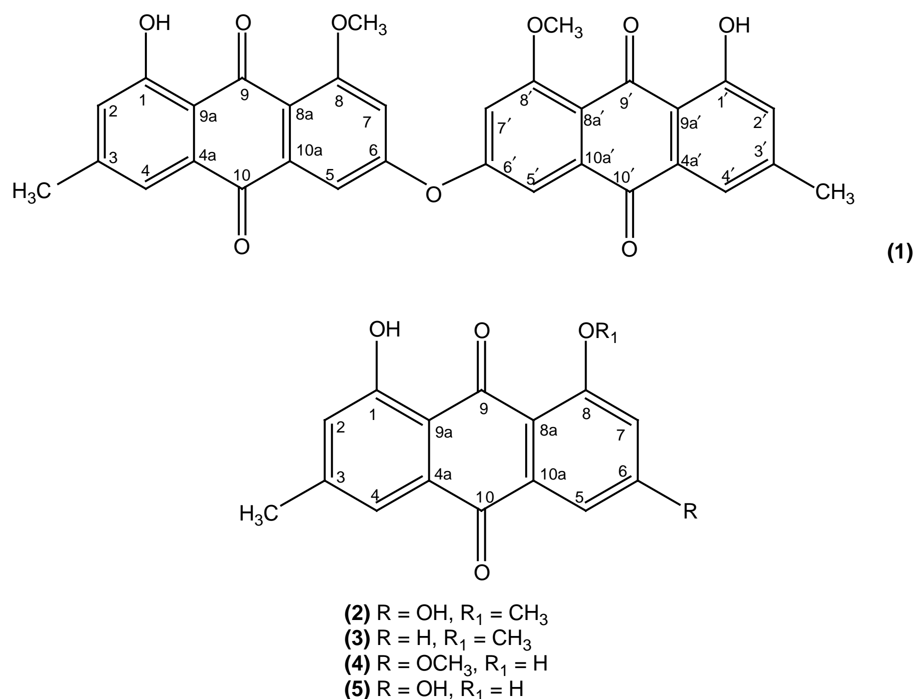

Figure 1. Structures of compounds **1**–**5**.

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of *Cassia artemisioides* (Gaudich. Ex. DC) Randell (Family Leguminosae).

2. Results and discussion

Compound **1**, as an orange-colored solid, gave pink color with ammonia vapors, indicating the presence of hydroxy anthraquinone moiety. FAB-MS (positive) showed a fragment ion at *m/z* 461.0 [M^+-91(H + $2xCH_3O$ + CO)]. IR absorption bands at 3340, 2912, 1680, and 1592 cm^{-1} indicated the presence of hydroxyl group, saturated CH, conjugated carbonyl, and aromatic ring (C═C), respectively. UV spectrum showed absorption maxima at 337, 328, 268, and 213 nm. ^1H and ^{13}C NMR spectral data are given in Table 1.

The ^{13}C NMR spectrum showed 22 signals (with some overlapping peaks), including 2 methyl, 2 methoxy, 8 methine, and 20 quaternary carbons. All the assignments were established from the DEPT experiments. The resonances showed similarity to those of 1,6-dihydroxy-8-methoxy-3-methyl anthraquinone (**2**) [17] except for C-6 and also most of the carbon signals were duplicated, which indicated that the compound was a symmetrical dimer of 1,6-dihydroxy-8-methoxy-3-methyl anthraquinone. The low-field signals at δ 186.9 and 186.8 were attributed to the chelated carbonyls at C-9 and C-9′, while the signal at δ 182.6 was assigned to the carbonyls C-10 and C-10′. The oxygen deshielded resonances at δ 164.5 were assigned to C-1 and C-1′, whereas the signal at δ 164.0 was assigned to C-8 and C-8′. The resonances at δ 162.6 and 162.3 were attributed to C-6 and C-6′, respectively.

The signals at δ 146.7, 146.6, and 137.6 were attributed to C-3, C-3′, and C-10a, respectively. The resonances at δ 137.6, 132.7, 132.6, and 124.1 were assigned to C-10a, C-4a, C-4′a, and C-2, respectively. The signals at δ 123.9, 119.0,

Table 1. ^{1}H and ^{13}C NMR spectral data in $(CD_3)_2CO$ of compound **1**.

No.	δ_C		δ_H (J_{HH} Hz)
1	164.5	C	13.33 (s, OH)
2	124.1	CH	7.07, s
3	146.7	C	–
4	119.0	CH	7.47, s
4a	132.7	C	–
5	107.0	CH	7.31 (d, $J = 2.2$)
6	162.6	C	–
7	104.9	CH	6.90 (d, $J = 2.2$)
8	164.0	C	–
8a	113.7	C	–
9	186.9	C	–
9a	114.8	C	–
10	182.4	C	–
10a	137.6	C	–
1′	164.5	C	13.33, s (OH)
2′	123.9	CH	7.07, s
3′	146.6	C	–
4′	119.0	CH	7.47, s
4′a	132.6	C	–
5′	106.9	CH	7.31 (d, $J = 2.2$)
6′	162.3	C	–
7′	104.9	CH	6.90 (d, $J = 2.2$)
8′	164.0	C	–
8′a	113.7	C	–
9′	186.8	C	–
9′a	114.8	C	–
10	182.4	C	–
10′a	137.6	C	–
3-CH_3	20.9	CH_3	2.41 (3H, s, CH_3)
3′-CH_3	20.9	CH_3	2.41 (3H, s, CH_3)
8-OCH_3	56.3	CH_3	3.95 (3H, s, OCH_3)
8′-OCH_3	56.3	CH_3	3.95 (3H, s, OCH_3)

119.0, and 114.8 were assigned to C-2′, C-4, C-4′, and C-9a, respectively. The signals at δ 114.8, 113.7, 113.7, and 107.0 were attributed to C-9′a, C-8, C-8a, and C-5, respectively. The resonances at δ 106.9, 104.9, and 104.9 were attributed to C-5′, C-7, and C-7′, respectively. The carbon signal at δ 56.3 was assigned to the two symmetrical methoxy groups attached at positions C-8 and C-8′. The signal at δ 20.9 was assigned to the two methyl groups attached to C-3 and C-3′.

^{1}H NMR spectrum displayed low-field signal at δ 13.33 (s, OH-1, OH-1′), which was assigned to the chelated hydroxy groups. The positions of hydroxyl groups were determined to be at C-1 and C-1′ from the HMBC techniques. The aromatic signal at δ 7.47 (2H, s) was assigned to H-4 and H-4′, and the *meta* coupled doublets for aromatic protons at δ 7.31 (2H, d, $J = 2.3$ Hz) were attributed to H-5 and H-5′. The singlet integrated for two protons at δ 7.07 (2H, s) was assigned to H-2 and H-2′. The *meta* coupled doublet at δ 6.90 (2H, d, $J = 2.3$ Hz), integrated for two protons, was attributed to H-7 and H-7′. The oxygen deshielded protons at δ 3.95 (6H, s) were assigned to the two methoxy groups at C-8 and C-8′. The upfield singlet at δ 2.41 (6H, s) was assigned to the two methyl groups at C-3 and C-3′. All the C—H correlations were achieved by using HMQC techniques.

The HMBC spectrum of compound **1** showed some interesting correlations. The OH proton at δ 13.3 showed correlations to C-1 and C-1′. The proton at δ 7.47 (H-4) showed correlations to the carbon resonances at δ 182.4 (C-10), 146.7 (C-3), 124.1 (C-2), and 114.8 (C-9a) (Figure 2). The

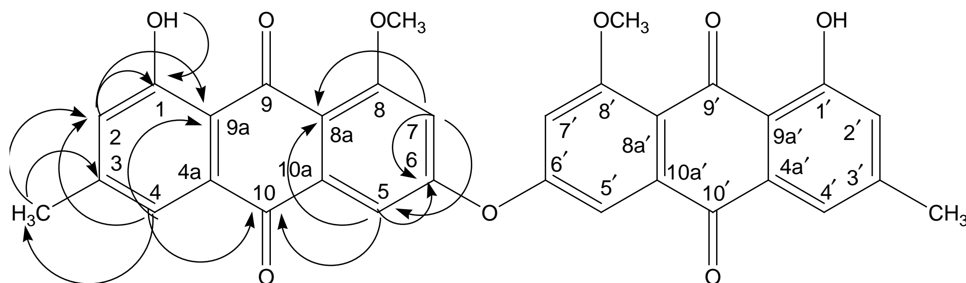

Figure 2. Key HMBC correlations of compound **1**.

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proton at δ 7.31 (H-5) correlated to the carbons at δ 182.4 (C-10), 162.6 (C-6), 113.7 (C-8a), and 104.9 (C-7). The proton resonance at δ 7.07 (H-2) showed correlations to the carbons at δ 119.0 (C-4), 114.8 (C-9a), and 146.7 (C-3). The signal at δ 6.90 (H-7) correlated to the resonances at δ 107.0 (C-5), 113.7 (C-8a), 162.6 (C-6), and 164.0 (C-8). The methoxyl protons at δ 3.95 showed correlations to C-8, and on the basis of HMBC correlation, the positions of methoxyl groups were assigned at C-8 and C-8′. The upfield chemical shift at δ 2.41 (CH_3) showed correlations to the carbons at δ 146.7 (C-3), 124.1 (C-2), and 119.0 (C-4).

On the basis of these spectral data, the structure of compound **1** was elucidated to be 1,1′-dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-*O*-bianthraquinone. To the best of our knowledge, it is a new natural product and is a dimer of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone.

Compounds **2**–**5** were identified as 1,6-dihydroxy-8-methoxy-3-methyl anthraquinone (**2**) [17], 1-hydroxy-8-methoxy-3-methylanthraquinone (**3**) [18], 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (**4**) [19], and 1,6,8-trihydroxy-3-methylanthraquinone (**5**) [20].

The isolated anthraquinones were evaluated for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity (% RSA) assay as summarized in Table 2. Generally, anthraquinones showed weak scavenging activity as compared to flavonoids. Among the isolated anthraquinones, only 1,6,8-trihydroxy-3-methylanthraquinone (**5**) showed comparatively a good activity, while others showed a weak activity. The activity of compound **5** may be attributed to the greater number of hydroxyl groups. Several studies concerning the relationship between the phenolic nature of flavonoids, phenols, and anthraquinones have been conducted [21]. Huang *et al.* [22] studied the relationship of anthraquinone derivatives and their antioxidant activities and concluded that hydroxyl groups either at *meta* or *para* positions are responsible for the antioxidant activity. It has also been reported that not only the hydroxyl group, but also the carbonyl group of anthraquinone skeleton is responsible for the antioxidant potential [23].

3. Experimental

3.1 General experimental procedures

Melting points were determined on a hot stage microscopic melting point apparatus and are uncorrected. Elemental analysis was done on EURO ER 300 (Italy). IR spectra were carried out using a Nicolet 380 FT-IR Spectrometer. Mass spectrum was obtained with a Finnigan MAT 312 double-focusing mass spectrophotometer, coupled with PDP 11/34 computer system. NMR spectra were acquired on JEOL ECA 600 spectrometers with solvent as an

Table 2. % RSA (DPPH) and EC_{50} values (μg/ml) of compounds **1**–**5**.

Compound	% RSA (100 μg/ml)	EC_{50} (μg/ml)
1,1′-Dihydroxy-3,3′-dimethyl-8,8′-dimethoxy 6,6′-*O*-bianthraquinone (**1**)	10.80	500.10
1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (**2**)	15.4	340.10
1-Hydroxy-8-methoxy-3-methylanthraquinone (**3**)	12.43	410.10
1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (**4**)	13.90	385.23
1,6,8-Trihydroxy-3-methylanthraquinone (**5**)	35.9	150.10
Standards		
Quercetin	98.2	4.12
Ascorbic acid	97.4	6.20

internal standard. Column chromatography (CC) was carried out using silica gel (E. Merck, Damstadt, Germany) (70–230 mesh), and thin layer chromatography (TLC) was performed using precoated silica gel on aluminum plates (E-Merck).

3.2 Plant material

The root bark of *Cassia aretemisioides* was collected in January 2006 from Peshawar district, identified by Prof. S.I. Ali of Karachi University. A voucher specimen number Kz-002 has been deposited in the herbarium of Jehanzeb Post Graduate College, Swat, Pakistan.

3.3 Extraction and isolation

The root barks of *C. aretemisioides* (0.2 kg) were soxhlet extracted with *n*-hexane followed by dichloromethane, ethyl acetate, and methanol, each for 8 h, followed by evaporation of the solvent under reduced pressure using a rotary evaporator. The ethyl acetate fraction (7.2 g) was subjected to CC on silica gel eluting with *n*-hexane and ethyl acetate mixture (starting at 9:1) as a solvent system in increasing order of polarity to afford 165 fractions. Similar fractions were combined to give three major fractions. Fraction 1 was subjected to CC eluted with hexane:ethyl acetate (9:1) followed by preparative. TLC using hexane:ethyl acetate (7:3) as mobile phase affording compounds 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (**2**) (11.5 mg) and 1-hydroxy-8-methoxy-3-methylanthraquinone (**3**) (11.5 mg). Fraction 2 on CC eluted with hexane:ethyl acetate (from 3:2 to 1:1) followed by preparative TLC using *n*-hexane:ethyl acetate (2:3) as eluting solvent, yielded compounds 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (**4**) (9.5 mg) and 1,6,8-trihydroxy-3-methylanthraquinone (**5**) (14.5 mg). Fraction 3 on CC eluted with hexane:ethyl acetate (2:3) yielded 1,1′-dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-*O*-bianthraquinone (**1**) (9.23 mg).

3.3.1 *1,1′-Dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-O-bianthraquinone (**1**)*

Orange red powder (methanol); melting point: 288–290°C; UV: λ_{max} 337, 328, 268, and 213 nm; IR ν_{max} (neat): 3340, 2912, 1592, 1485, 1380, 1250 cm^{-1}; ^{1}H and ^{13}C NMR spectral data are given in Table 1; FAB (Positive) *m/z* 461.0 [M^{+}-91(H + 2xCH_3O + CO or H + 2xOH + 2xCO), 425.0, 369.0, 333.0, 277.0, 259.0, 241.0, 185.0, 167.0, 149.0, 117.0. Elemental analysis: Found: C, 69.12%, H, 4.35%; calcd for $C_{32}H_{22}O_9$: C, 69.81%, H, 4.00%.

3.4 Antioxidant activity as DPPH radical-scavenging assay

The RSAs of the pure compounds and standards were measured from the bleaching of the purple-colored methanol solution of DPPH using a slightly modified method of Blois [24] and Yildirim *et al.* [25]. Briefly, 1 ml of 1 mM solution of DPPH in methanol was mixed with 3 ml of the sample solutions in ethanol (containing 20–100 μg) and control (without the sample). After 30 min, the absorbance at 517 nm was measured. An increase in the DPPH RSA is correlated with the decrease in the absorbance. The percentage of scavenging activity (% RSA) of DPPH was calculated as follows:

$$\%RSA = \frac{\text{Control absorbance} - \text{sample absorbance} \times 100}{\text{Control absorbance}}.$$

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The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. The EC_{50} (compound concentration showing 50% inhibition) was calculated from the graph of % RSA against compound concentration using quercetin and ascorbic acid as standards.

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