ORIGINAL ARTICLE

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Rec. Nat. Prod. 9:2 (2015) 169-174

records of natural products

Peshawaraquinone a Novel Naphthoquinone and a New Indanone from the stem of *Heterophragma adenophyllum* Seem

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(Received September 27, 2013; Revised March 19, 2014; Accepted September 20, 2014)

Abstract: Peshawaraquinone; a novel naphthoquinone **(1)** and a new indanone **(2)** were isolated from the stem heartwood of *Heterophragma adenophyllum* Seem. Their structures were elucidated using advance spectral techniques including X-ray crystallography for the compound **1**.

Keywords: Bignoniacea; *Heterophragma adenophyllum* Seem; peshawaraquinone; indanone.

1. Introduction

Heterophragma adenophyllum Seem (Family Bignoniacea) is distributed in Southeast Asia and Africa as an ornamental tree with white fissured bark [1]. It is extensively used in folk medicine for various ailments like antidiabetic, amenorrhoea, premature ejaculation, night emission, antimicrobial activity, antifungal activity, antiseptic activity and skin disease [2]. Previous chemical investigations led to the isolation of lapachol, dehydro-α-lapchone, α-lapachone, β-amyrin, adenophyllone, tecomoquinone-I, dilapachone, β-sitosterol and dehydro-iso-α-lapchone [1]. Lapachol (2-hydroxy-3-(3′-methyl-2′-butenyl)-1,4-naphthoquinone) is present in almost all the species of Bignoniacea. A wide range of biological activities has been demonstrated by lapachol and its transformations including the following: anti-abscess, anti-edemic, anti-inflammatory, anti-ulcer, anti-carcinomic, anti-malarial, anti-viral, anti-leshmanial, fungicidal, pesticidal, respiradepressant, protisticidal, schistosomicidal, viricidal and termiticidal [3-9]. Now we report a novel naphthoquinone (peshawaraquinone) **(1)** and a new indanone **(2)** (Figure 1).

2. Materials and Methods

2.1. General experimental procedure

The melting points were determined on a Stuart digital melting point (SMP 10) apparatus. Ultraviolet-visible (UV-Vis) spectra were recorded in chloroform using an Optima UV-Visible spectrophotometer. IR spectra were recorded on a Shimadzu FTIR Prestige-21 spectrophotometer. Mass spectra were carried out on a JEOL MS Route Direct Probe. The ^{1}H, ^{13}C NMR and HMBC studies were carried on a Bruker Avance-AV- 400 and 100 MHz**.** 2D studies were carry out using Topspin software**.** The XRD was carried out at Department of Physics, University of Sargodha,

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 01/01/2015 EISSN:1307-6167

Pakistan using Bruker Kappa APEXII CCD diffractometer. All commercial grade solvents were distilled before use for extraction and column chromatography.

2.2. Plant material

The stem heartwood of *Heterophragma adenophyllum* Seem was collected from the University of Peshawar, Khyber Pukhtunkhwa, Pakistan in April 2011, identified in the Botany Department, University of Peshawar where its voucher specimen (Bot-20020 pup) is deposited in the herbarium.

2.3. Extraction and Isolation

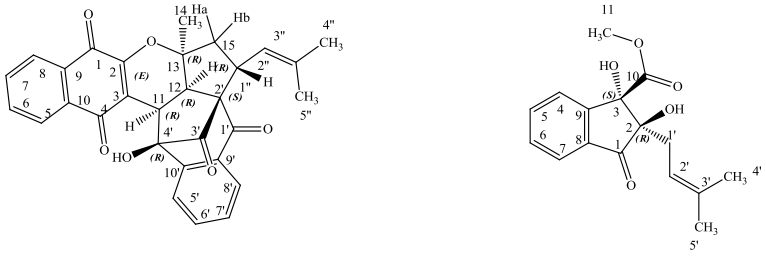
The powdered air dried stem heartwood (200 g) was extracted with methanol for 6 hour in a soxhlet apparatus and the solvent was evaporated under reduced pressure. The crude extract (25 g) so obtained was subjected to subsequent fractionation and evaporation in the order of increasing polarity using the solvents (yield): hexane (1.0 g), dichloromethane (2.0 g), ethyl acetate (1.5 g) and methanol (12.0 g). The methanol fraction (12.0 g) was subjected to column chromatography (CC) on silica gel (70-230 mesh) using a mixture of chloroform:methanol (10:0→0:10) as a mobile phase. A total of 114 fractions so obtained were compiled (TLC profile) into three major fractions: **1** (10.0→9:1, 500 mL, 2.2 g), **2** (9:1→7:3, 1000 mL, 5 g) and **3** (7:3→4:6, 1000 mL, 3.5 g). Fraction **2** (5 g) was eluted on column chromatography using chloroform and methanol (10:0→7:3) yielded two sub fractions: **2.1** (10.0→9:1, 500 mL, 1.5 g) and **2.2** (9:1→7:3, 1000 mL, 3 g). The fraction **2.2** was subjected to column chromatography chloroform:methanol (8:2) to afford compound **1** (8 mg). Fraction **3** (3 g) was subjected to column chromatography on silica gel using chloroform:methanol (10.0→4:6) solvent yielded three sub-fractions: **3.1** (10.0→8:2, 200 mL, 0.5 g) **3.2** (8:2→5:5, 500 mL, 2 g) and **3.3** (5:5→4:6, 200 mL, 0.5 g). The sub-fraction **3.2** (2 g) on silica gel column chromatography using chloroform:methanol (6:4) yielded compound **2** (4 mg).

Peshawaraquinone (1**)**

Yellow crystalline solid, m.p.: 250-252 °C. HR-ESIMS: 480.1594 $[M-H]^+$ (Calculated for $C_{37}H_{39}N_2O_7$ = 480.16). UV ($CHCl_3$) λ_{max} (log ε): 250 (6.99), 270 (5.5), 280 (5.61) nm. IR ῡ: 3441.01 (O-H, st.), 1760.80, 1680, 1680.1, 1680.2 (C=O, carbonyl st.) cm^{-1}. ^1H and ^{13}C-NMR: See Table 1.

Indanone (Methyl-1,2-dihydroxy-2-(3-methylbut-2-en-1-yl)-3-oxo-2,3-dihydro-1H-indene-1-carboxylate) (2)

Colourless crystalline compound, m.p.: 220-221°C, (Lit. 220-221 °C) [10]. HR-ESIMS: 290.133 $[M H]^+$ (Calculated for $C_{16}H_{18}O_5$ = 290.12). UV ($CHCl_3$) λ_{max} (log ε): 230 (5.27), 240 (5.29) nm. IR ῡ: 3379.2 (O-H, st.), 1724.3, 1725.5 (C=O, carbonyl st.) cm^{-1}. ^1H and ^{13}C-NMR: See Table 2.



(1) **(2)**

Figure 1. Structure of peshawaraquinone **(1**) and methyl-1,2-dihydroxy-2-(3-methylbut-2-en-1-yl)-3- oxo-2,3-dihydro-1H-indene-1-carboxylate **(2)**

3. Results and Discussion

3.1. Structure elucidation

From the methanol extract (12 g) of the stem heartwood of *Heterophragma adenophyllum* Seem, were isolated two new and three known compounds; a novel naphthoquinone we named as peshawaraquinone **(1)**, a new indanone; methyl-1,2-di-hydroxy-2-(3-methylbut-2-en-1-yl)-3-oxo-2,3-dihydro-1H-indene-1-carboxylate **(2)** and three known compounds; lapachol, α-lapachone and dehydro-α-lapachone. All the structures were elucidated using various spectroscopic techniques (IR, UV, ^{1}H and ^{13}C-NMR, HSQC, COSY, HMBC, NOESY, MS). Peshawaraquinone **(1)** was further confirmed by X-ray crystallography.

Peshawaraquinone **(1)**. The ^{1}H and ^{13}C NMR (Table 1) resonances and three bonds COSY (^{1}H-^{1}H) correlations of compound **1** were characteristics of naphthoquinone dimer. In ^{13}C NMR the four carbonyls were resonating at δ_C 184.4 (C-1), 179.3 (C-4), 193.5 (C-1′) and 203.6 (C-3′). According to the COSY (^{1}H-^{1}H) spectra, the four methines of the two benzene ring (each) were having ABCD orientation which appeared in the ^{1}H and ^{13}C NMR at δ_H 8.12 (1H, dd, J = 8.4, 1.6 Hz, H-5), 7.74 (1H, m, H-6), 7.74 (1H, m, H-7), 8.16 (1H, dd, J = 8.4, 1.6 Hz, H-8), 7.92 (1H, dd, J = 8.0, 1.5 Hz, H-5′) 7.74 (1H, m, H-6′), 7.51 (1H, m, H-7′), 8.04 (1H, dd, J = 7.6, 1.5 Hz, H-5′); δ_C 126.7 (C-8), 133.6 (C-6), 134.3 (C-7), 126.7 (C-8), 124.8 (C-5′), 135.5 (C-6′), 129.1(C-7′) and 127.6 (C-8′). The aromatic quaternary carbons were resonating at δ_C 131.9 (C-9), 131.1 (C-10), 129.7 (C-8′) and 144.6 (C-10′). The olefinic quaternary carbons were resonating at δ_C 120.7 (C-2), 154.9 (C-3) and 133.9 (C-3″). The methine resonating at δ_H 3.82 (1H, d, J = 10.4 Hz, H-11), 2.76 (1H, d, J = 10.4 Hz, H-12); δ_C 35.5 (C-11), 53.6 (C-12) were COSY (^{1}H-^{1}H) correlated with each other (Table 1). Similarly, the diastereotropic methylene resonating at δ_H 2.62 (1Ha, dd, J = 13.2, 6.4 Hz, Ha-15) and 2.17 (1Hb, t, J = 13.2 Hz, Hb-15); δ_C 48.7 (C-15) were COSY (^{1}H-^{1}H) correlated with the methine resonating at δ_H 3.78 (1H, m, H-1″); δ_C 36.8 (C-1″) which in turn showed COSY (^{1}H-^{1}H) correlation with the methine resonating at δ_H 5.94 (1H, d, J = 9.6 Hz, H-2″); δ_C 122.4 (C-2″). The two geminal methyls attached to the double bond were resonating at δ_H 1.76 (3H, s, H-4″) and 1.75 (3H, s, H-5″); δ_C 18.1 (C-4″) and 26.3 (C-5″) while the methyl attached to the oxygenated quaternary carbon was resonating at δ_H 1.30 (3H, s, H-14); δ_C 21.5 (C-14). The saturated quaternary carbon was resonating at δ_C 120.7 (C-2) while the two quaternary carbons (oxygenated) were resonating at δ_C 85.8 (C-4′) and 87.1 (C-13).The hydroxyl proton was resonating at δ_H 3.54 (1OH, s). The aromatic protons; H-8 and H-5 showed three bond HMBC correlations with the naphthoquinone carbonyls C-1 and C-14 respectively while the aromatic protons; H-8′ and H-5′ showed three bond HMBC correlations with the carbonyl C-1′ and hydoxylated carbon; C-4′ respectively. The pyran protons H-11 and H-12 showed three bonds HMBC correlations with the hydoxylated carbon C-4 and carbonyl C-1′ respectively. These correlations established a dimeric naphthoquinone skeleton attached through the pyran moiety.The diastereotropic methylene protons; Ha-15 and Hb-15 showed HMBC correlations with the carbons of pyran nucleus; C-12, C-13 and the carbons of cyclopentane nucleus; C-2′ and C-1″. The protons of the two methyl groups H-4″ and H-5″ showed HMBC correlations with each other (C-4″ and C-5″) as well as with the methine C-2″. The methine proton H-2″ showed three bonds HMBC coupling with the methylene carbons C-15 and quaternary carbon C-2″. These correlations verify the attachment of 2-methyl 1-propenyl group at methine C-1″. The methyl protons H-14 showed three bonds HMBC correlations with the pyran methine C-12 and methylene C-15 which confirmed the attachment of CH_3-14 at the pyran carbon C-13. The methine proton H-1″ showed three bonds correlation with the pyran methine C-12, carbonyl C-1′ and olefinic carbon C-3″. These correlations established the attachment of cyclopentane ring with the pyran ring at carbons C-12 and C-13. The hydroxyl proton OH-4′ showed three bonds HMBC correlations with the pyran methine C-11, carbonyl C-3′ and aromatic carbon C-10′. These correlations showed the presence of cycloheptanone ring bridgehead at carbons; C-2′ and C-4′ resulted into bicyclo (cyclohexanone and cyclopentanone) attached with the pyran ring at carbons C-11 and C-12 and cyclopentane ring at carbon C-12 and C-2.

The OH-4′ proton showed NOESY correlation with protons of the methyl group while the protons of the pyran ring H-11 and H-12 showed correlations with each other as well as with the protons of the cyclopentane ring H-1″. The NOESY (Figure 3) correlations were further confirmed

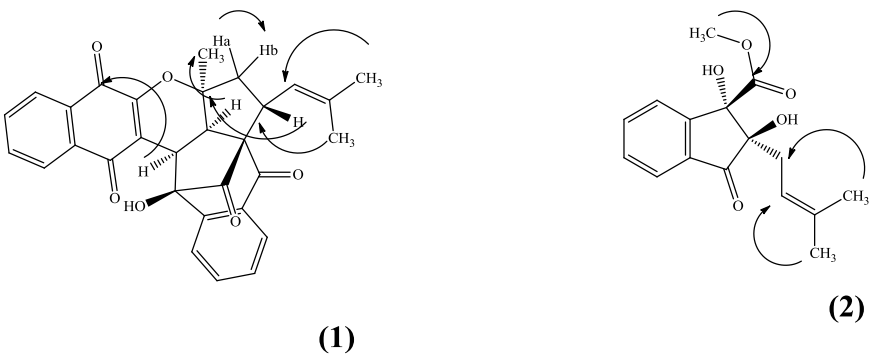
through XRD data analysis. So, the absolute stereochemistry of Peshawaraquinone (**1**) is given in Figure 1 while its 3D XRD is given in Figure 4. The IUPAC name of Peshawaraquinone (**1**) is (5aR,5a1R,6R,7aS,14bR,15R)15-hydroxy-7a-methyl-6-(2-methylprop-1-en-1-yl)-7,7a,14b,15-tetrahydro-5H-t-5a,15methanobenzo[g]benzo[5,6]azuleno[1,8-bc]chromene-5,9,14,16(5a1H,6H)-tetraone.

The important COSY and HMBC correlations of Peshawaraquinone **(1) a**re given in Figure 3 and 2 respectively.

Indanone (**2**). The ^{1}Hand ^{13}C NMR (Table 2) resonances and three bonds COSY (^{1}H-^{1}H) correlations of compound **2** were characteristics of indanone skeleton substituted with methyl carboxylate and 2-methyl-2-butene moieties. According to the COSY (^{1}H-^{1}H) spectra, the methine of the benzene ring were having ABCD orientation which appeared in the ^{1}H and^{13}C NMR at δ_H 7.80 (1H, dd, J = 7.6, 1.6 Hz, H-4), 7.54 (1H, m, H-5), 7.70 (1H, m, H-6) and 7.62 (1H, dd, J = 7.6, 1.6 Hz, H-7); δ_C 123.4 (C-4), 130.1 (C-5), 135.4 (C-6) and 124.2 (C-7).The two aromatic quaternary carbons were resonating at δ_C 148.4 (C-8), 134.9 (C-9).The diastereotropic methylene was resonating at δ_H 2.78 (1Ha, dd, J = 14.8, 8.4 Hz, Ha-1′) and 2.10 (1Hb, dd, 14.8, 7.2 Hz, Hb-1′); δ_C 33.1 (C-1′), coupled with the methine resonating at δ_H 5.07 (1H, dd, J = 8.4, 7.2 Hz, H-2′); δ_C 116.3 (C-2′). The two geminal methyls were resonating at δ_H 1.60 (3H, s, H-4′) and 1.50 (3H, s, H-5′); δ_C 25.9 (C-4′) and 17.9 (C-5′) while the methoxy methyl was resonating at δ_H 3.66 (3H, s, H-11); δ_C 53.8 (C-11). The two hydroxyl protons were resonating at δ_H 3.00 (1OH, s) and 4.35 (1OH, s). The two carbonyls (ketone and ester respectively) were resonating at δ_C 201.9 (C-1) and 172.8 (C-10). The two hydoxylated quaternary carbons were resonating at δ_C 83.1(C-2) and 87.3 (C-3) while the olefinic quaternary carbon was resonating at δ_C 137.7 (C-3′). The aromatic proton H-8 showed three bond HMBC correlations with the aromatic carbon C-6 and hydoxylated carbon C-3. Similarly, the aromatic proton H-7 showed three bond HMBC correlations with the aromatic carbon C-5 and carbonyl C-1. The hydroxyl proton OH-3 showed three bond HMBC correlations with aromatic carbon C-9, ester carbonyl C-10 and hydoxylated carbon C-2. Similarly, the hydroxyl proton OH-2 showed three bond HMBC correlation with carbonyl (ketone) C-1, hydoxylated carbon C-3 and methylene C-2′. The correlation confirmed the indanone skeletons substituted with methyl carboxylate at carbon C-3 and two hydroxyl groups at carbons C-2 and C-3. The protons of the two geminal methyls H-4′ and H-5′ are HMBC correlated with each other (C-4′ and C-5′) and the methine C-2′. The diastereotropic protons 2H-1′ have three bonds HMBC correlations with the carbonyl C-1, olefinic carbon C-3′ and hydoxylated carbon C-3. These correlations confirmed the presence of 2-methyl-2-butene moiety attached at carbon C-2 of the indanone ring. The proton H-4 showed NOESY correlation (Figure 3) with the protons H-2′. The hydroxyl proton OH-2 showed correlation with proton H-7. The two hydroxyl protons have no NOESY correlation with each other.

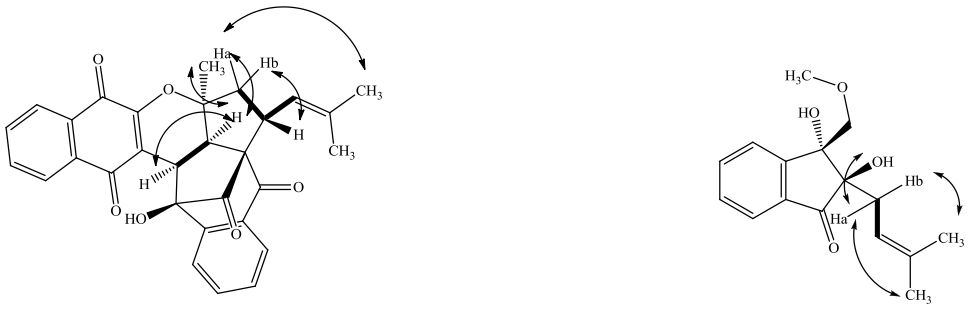
The relative stereochemistry was confirmed by comparing with the literature [10] and was assigned as (*1S, 2R*) methyl-1,2-di-hydroxy-2-(3-methylbut-2-en-1-yl)-3-oxo-2,3-dihydro-1H-indene-1-carboxylate **(2)** (Figure 1).

To the best of our knowledge indanone **2** is synthetically prepared [10] having only X-ray crystallography data but we are reporting it for the first time as a natural product along with all the spectral data including IR, UV, ^{1}H NMR, ^{13}C NMR, HMBC and MS.



(1) **(2)**

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



(1) **(2)**

Figure 3. Key NOESY (⟷) and COSY (▬) correlations of compound **(1)** and **(2)**

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data for peshawaraquinone (**1**) in $CDCl_3$

C. No.	^{13}C (δ)	^{1}H (δ) (*J* in Hz)		C. No	^{13}C	^{1}H (δ) (J_{HH} Hz)
1	184.4			1′	193.5	
2	120.7			2′	73.4	
3	154.9			3′	203.6	
4	179.3			4′	85.8	
5	126.7	8.12 (dd, 8.4, 1.6)	5′	124.8		7.92 (dd, 8.0, 1.5)
6	133.6	7.74 (m)	6′	135.5		7.74 (m)
7	134.3	7.74, (m)	7′	129.1		7.51 (m)
8	126.7	8.16 (dd, 8.4, 1.6)	8′	127.6		8.04 (dd, 7.6, 1.5)
9	131.9			9′	129.7	
10	131.1			10′	144.6	
11	35.5	3.82 (d, 10.4)		1″	36.8	3.78 (m)
12	53.6	2.76 (d, 10.4)		2″	122.4	5.94 (d, 9.6)
13	87.1			3″	133.9	
14	21.5	1.30 (s)		4″	18.1	1.76 (s)
15	48.7	2.62, (dd, 13.2, 6.4)	5″	26.3		1.75 (s)
		2.17 (t, 13.2)	1OH			3.54 (s)

Table 2. ^{1}H NMR and ^{13}C NMR spectroscopic data for indanone **2** in $CDCl_3$

C. No.	^{13}C (δ)	^{1}H (δ) (*J* in Hz)	C. No.	^{13}C (δ)		^{1}H (δ) (*J* in Hz)
1	201.9		10	172.8		
2	83.1		11	53.8		3.66 (s)
3	87.3		1′	33.8		2.78 (dd, 14.8, 8.4)
4	123.4	7.80 (dd, 7.6, 1.6)				2.10 (dd, 14.8, 7.2)
5	130.1	7.54 (m)	2′	116.3		5.07 (dd, 8.4, 7.2)
6	135.4	7.70 (m)	3′	137.7		
7	124.2	7.62 (dd, 7.6. 1.6)	4′	25.9		1.60 (s)
8	148.4		5′	17.9		1.50 (s)
9	134.9		2OH	3.00 (s)		4.35 (s)

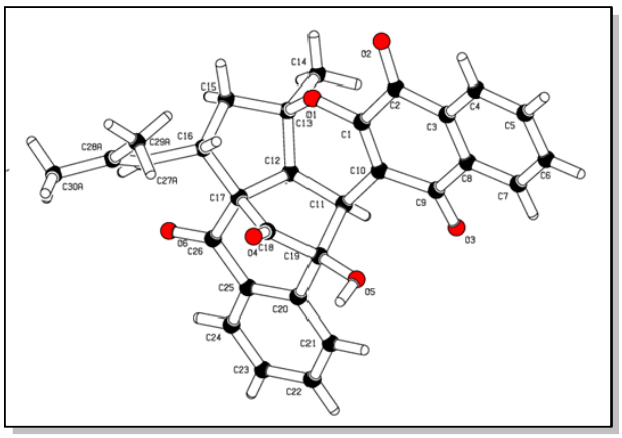


Figure 4. X-ray crystallography of peshawaraquinone **(1)**

3.3 Antimicrobial activity

Peshawaraquinone **(1)** was evaluated against few bacterial strains; *Staphylococcus aureus, Klebsiella pneumonia* and *Staphylococcus epidermidis* showing no significant activity.

Acknowledgments

The authors are thankful to HEJ Research Institute, University of Karachi, Pakistan for performing NMR and mass spectroscopic techniques.

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