Isolation and characterisation of three new anthraquinone secondary metabolites from *Symplocos racemosa*

Umar Farooq\textsuperscript{a,}\textsuperscript{*}, Sadia Naz\textsuperscript{a}, Ajmal Khan\textsuperscript{a,}\textsuperscript{*}, Sara Khan\textsuperscript{a}, Afsar Khan\textsuperscript{a,}\textsuperscript{*}, Mumtaz Ali\textsuperscript{b} and Saleha Suleman Khan\textsuperscript{c}

\textsuperscript{a}Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan; \textsuperscript{b}Department of Chemistry, University of Malakand, Chakdara, Dir (L), Pakistan; \textsuperscript{c}H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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Three new anthraquinone secondary metabolites were isolated from *Symplocos racemosa*, a small tree of family symplocaceae. The structures of compounds (1–3) were elucidated to be 1,4-dihydroxy-6-(ethoxymethyl)-8-propylanthracene-9,10-dione (1), 1,4-dihydroxy-6-(hydroxymethyl)-8-butylanthracene-9,10-dione (2) and 1,4-dihydroxy-6-(hydroxymethyl)-8-propyl anthracene-9,10-dione (3) using their spectral data, i.e. through IR, UV, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and two-dimensional (2D) NMR techniques including heteronuclear multiple quantum coherence, heteronuclear multiple bond correlation and correlation spectroscopy.

**Keywords:** anthraquinone; *Symplocos racemosa*; Symplocaceae

1. Introduction

*S. racemosa* is an evergreen plant or shrub belonging to family symplocaceae and has been used by local practitioners for treatment of different diseases including diarrhoea, dysentery, liver complaints, uterine disorders in dropsy, scorpion stings and eye diseases (Ahmad et al. 2005; Devmurari 2010). *S. racemosa* has been used as a remedy for gynaecological disorder in Ayurvedic medicine system. It has been used as a single drug or in multicomponent preparations, for example, Lodhrasava for disorders in female reproductive system. Its use in treatment of gum disorders like spongy gums and bleeding has also been reported (Ahmad et al. 2003). *S. racemosa* has been reported scientifically as an anticancer, antimicrobial and anti-inflammatory agent (Kumar et al. 2007; Kambhoja & Keshava Murthy 2010). The ethyl acetate fraction of *S. racemosa* is an effective hepatoprotective agent for...
hepatic damage in rats induced by CCl₄ and can be used for treatment of liver damage (Wakchaure et al. 2011).

Various chemical constituents like glycosides including symposide, symplcoside, leucopelargonidin-3-glucose, flavonol glycoside, 28-O-bis-β-glucopyranosides, β-amyrin phenolic glycosides and C-glycoside symcososide have been isolated from S. racemosa. Similarly, isoloturine, alkaloid loturine, saliperin, symplcruconic acid and symptecmoside were isolated from S. racemosa (Ahmad et al. 2006, 2007; Badoni et al. 2010).

In this study, we describe the isolation of three new anthraquinone analogues isolated from S. racemosa and detailed characterisation of these compounds was carried out through IR, UV, ¹H NMR, ¹³C NMR and 2D NMR spectroscopic techniques.

2. Results and discussion

The ethyl acetate fraction of S. racemosa was subjected to repeated chromatography, which resulted in the isolation of three new anthraquinone analogues (1–3) (Figure 1), and their structures were elucidated by spectroscopic techniques and comparison with the literature.

Compound 1 was isolated as a pink gummy solid and its molecular formula was found to be C₂₀H₂₀O₅ as suggested by the highest peak of m/z 340.1320 in HR-EI-MS, and m/z (%) values obtained were 310.7 (47), 296.5 (41), 312.1 (25), 303.7 (29) and 293.6 (21). The UV spectrum showed bands at λ_max 472 (2.8), 281 (3.1), 232 (5.1) nm, suggesting an anthraquinone chromophore (Wu et al. 2014). The IR spectrum showed absorption bands at 3437, 2986, 1699 and 1180 cm⁻¹, indicating the presence of a hydroxyl group, an alkyl moiety and carbonyl stretching, respectively.

The ¹H NMR spectrum of compound 1 demonstrated that two aromatic protons at chemical shift (δ_H) values of 7.20 (1H, d, J = 9.5 Hz) and 7.16 (1H, d, J = 9.5 Hz) showed ortho coupling to each other (Table S1). Meta coupling was observed between the aromatic protons at chemical shift (δ_H) values of 8.10 (1H, d, J = 2.1 Hz) and 7.52 (1H, d, J = 2.1 Hz). The ¹H NMR spectrum also revealed the presence of a propyl moiety having two methylene groups at (δ_H) 3.20 (2H, t, J = 7.9 Hz) and 1.75 (2H, m), while a methyl group appeared at (δ_H) 1.10 (3H, t, J = 7.7 Hz). Additionally, ¹H NMR spectrum showed the presence of ethoxy methyl group with chemical shift values for two methylene groups of (δ_H) 4.85 (2H, s) and 3.80 (2H, q, J = 6.8 Hz) along with one methyl group with a chemical shift value of (δ_H) 1.30 (3H, t, J = 7.7 Hz) (Table S1). The ¹³C NMR spectrum showed the presence of twenty carbon atoms comprising four CH₂, ten quaternary carbons, two methyl groups and four methine carbons. The ¹³C NMR spectrum showed the presence of two carbonyl carbons at (δ_C) 190.1 and 189.1 while other quaternary carbons resonated at (δ_C) of 118.6, 119.1, 128.1, 140.1, 149.4 and 156.1 (Table S2). Two down-field signals resonated at (δ_C) 162.7 and 162.1 due to the presence of two hydroxy groups and two methyl groups centred at (δ_C) 19.4 and 20.6 (Wu et al. 2014).

The HMBC (heteronuclear multiple bond correlation) and COSY (correlation spectroscopy) spectra were quite supportive for accurate placement of the substituent on anthraquinone ring. The COSY spectrum showed a strong correlation between H-2 and H-3. Similarly, a correlation between H-2" and H-3" of the ethoxy methyl substituent and three aliphatic protons of propyl group, i.e. H-1/ H-2 and H-2/H-3, were also observed (Figure S1).

The HMBC spectrum showed a correlation of H-2 (δ_H 7.20) with C-1 and C-3, while H-3 showed a strong correlation with C-2 and C-1. The HMBC correlation of H-5 (δ_H 8.10) with C-6, C-10a and C-10, while that of H-7 (δ_H 7.52) with C-6 and C-8 were also shown as depicted in Figure S1. Finally, the correlation of H-1" (δ_H 4.85) with C-2", C-5, C-6 and C-7 confirmed the position of the ethoxy methyl substituent. HMBC was quite supportive in the position of side aliphatic chains at C-6 and C-8. The HMBC correlation was shown to have connectivity of H-1'
(δH 3.20) with C-8, C-8a, C-2′ and C-3′ and, based on all these facts, the structure was proposed to be 1,4-dihydroxy-6-(ethoxymethyl)-8-propylanthracene-9,10-dione (1).

Compound 2 was isolated as a brown gummy solid from ethyl acetate fraction. The HR-EI-MS showed a molecular ion peak at m/z 326.1159 and the molecular formula was assigned as C19H18O5. The m/z (%) values obtained were 283.8 (35), 298 (29), 295.0 (21) and 291.0 (23). The IR spectrum showed peaks at 3390, 3406, 2910 and 1696 cm⁻¹, indicating the presence of a hydroxyl group, an alkyl group and carbonyl moieties in compound 2. The UV spectrum revealed absorption bands at λmax 472 (3.0), 274 (3.3) and 235 (5.4) nm, suggesting the presence of anthraquinone chromophore (Wu et al. 2014).

The 1H NMR and 13C NMR were quite similar to that of compound 1 as given in Table S1, and the only difference observed was the presence of a butyl moiety with chemical shift values of δH 3.18 (2H, t, J = 8.4; δC 39.1), δH 1.68 (2H, m; δC 36.4) and δH 1.52 (2H, m; δC 28.1) for three methylene groups, and CH₃ protons appeared at δH 1.12 (3H, t, J = 7.6; δC 19.3) for a methyl group (Table S1–S2). The position allocated to a butyl group at C-8 was supported by HMBC. H-1′ (δH 3.18) showed HMBC correlations with C-8 (δC 155.1), C-8a (δC 151.4), C-2′ (36.4) and C-3″ (28.1) and COSY correlations with H-1′/H-2′0, H-2′/H-3′ and H-3′/H-4′ (Figure S1). Additionally, downfield methylene appeared at H-1″ δH 5.18 due to the presence of a hydroxyl group revealed by 1H NMR spectrum. H-1″ showed cross peaks with C-6 (δC 151.4), C-7 (δC 136.5) and C-5 (δC 123.2), locating this substituent at C-6. As a result, the structure deduced from all spectral data and comparison with the literature (Wu et al. 2014) was 1,4-dihydroxy-6-(hydroxymethyl)-8-butyl anthracene-9,10-dione (2).

Compound 3 was isolated as a gummy solid and assigned the molecular formula of C18H16O5 as suggested by HR-EI-MS with a molecular ion peak at m/z 312.0105 and values of m/z (%) were 280.9 (31), 269.7 (27) and 276.9 (23). The UV spectrum showed absorption bands at 474 (2.7), 270 (3.1) and 234 (5.2) nm, while IR spectrum showed peaks at 3480 br, 1682 and 2975 cm⁻¹, showing the presence of a hydroxyl group, a carbonyl group and an alkyl functional group, respectively.
The $^1$H NMR and $^{13}$C NMR data of compound 3 were quite identical to compounds 1 and 2. The only difference observed was the presence of a propyl group at position 8 with chemical shift values of $\delta_H$ 3.21 (2H, t, $J = 8.2$; $\delta_C$ 44.1) and 1.82 (2H, m; $\delta_C$ 29.1) for two methylene groups and of $\delta_H$ 1.18 (3H, t, $J = 8.1$; $\delta_C$ 20.1) for a methyl group (Table S1–S2). The HMBC spectrum showed a correlation between H-1 (δ$_H$ 3.21) and C-8, C-8a and C-2; similarly, H-2 (δ$_H$ 5.10) showed HMBC cross peaks with C-5, C-6 and C-7 confirming the position of the propyl and hydroxy methyl substituents, respectively. The structure proposed from all spectral data was 1,4-dihydroxy-6-(hydroxymethyl)-8-propyl anthracene-9,10-dione (3).

3. Experimental part

3.1. General experimental procedure

IR and UV spectra were recorded using Hitachi JASCO-320-A (Tokyo, Japan) and Hitachi UV-3200 spectrophotometers (Tokyo, Japan), respectively, and double focusing Varian MAT-312 spectrometer (Japan) was used for EI-MS and HR-EI-MS. The Bruker AMX-500 MHz spectrometer (France) was used to record $^1$H NMR, $^{13}$C NMR and for 2D NMR spectra. Scalar coupling ($J$) was reported in Hz and chemical shift in parts per million ($\delta$) relative to tetramethyl silane. TLC was performed on precoated silica gel G-25-UV254 plates and values were detected at 254 nm and by ceric sulphate in 10% H$_2$SO$_4$ solution. While column chromatography was carried out on silica gel (E. Merck, 230–400 mesh and 70–230 mesh, E-Merck, Karachi, Pakistan).

3.2. Plant material

The plant S. racemosa (Symplocaceae) was collected from Hazara division of Khyber Pakhtunkhwa, Pakistan, in April 2013, and identified by Dr Manzoor Ahmad (Taxonomist) at the Department of Botany, Post-Graduate College, Abbottabad, Pakistan. A voucher specimen (no. 6453) has already been deposited at the herbarium of the Department of Botany, Post-Graduate College, Abbottabad, Pakistan.

3.3. Extraction and isolation

The whole plant material (8 kg) was collected from Hazara division of Khyber Pakhtunkhwa, Pakistan. Then, it was shade dried, ground into powder and soaked in methanol for extraction. The filtrate was subjected to a vacuum rotary evaporator to get crude extract (300 g). Four fractions namely n-hexane (80 g), chloroform (40 g), ethyl acetate (35 g) and n-butanol (86 g) were obtained through fractionation of crude extract. Ethyl acetate fraction was selected for further investigation on the basis of TLC.

3.4. Column chromatography

Ethyl acetate fraction was subjected to column chromatography using silica gel and n-hexane was used as a gradient of ethyl acetate up to 100% followed by methanol, which resulted in sub-fractions depending on the polarity of compounds. Sub-fractions no. 6–8 out of the total of 14, were re-subjected to recolumn chromatography to get compound 1 (10 mg) at EtOAc:hexane (20:80), while compound 2 (8.9 mg) and compound 3 (8.5 mg) were purified from sub-fractions no. 10–14 at EtOAc:hexane (35:65) and 33:67, respectively.
3.5. Characterisation of compound 1

Pink gummy solid: UV $\lambda_{\text{max}}$: 472 (2.8), 281 (3.1), 232 (5.1) nm. IR $v_{\text{max}}$ (KBr): 3430–3443, 2986, 1699, and 1180 cm$^{-1}$. EI-MS $m/z$: (rel. int.) 340 [M]+(100), 310.7 (47), 296.5 (41), 312.1 (25), 303.7 (29), 293.6 (21). HR-EI-MS: $m/z$ [M]+ calculated for C$_{20}$H$_{20}$O$_5$ 340.1311, found 340.1320.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.20 (1H, d, $J = 9.5$), 7.16 (1H, d, $J = 9.5$), 8.10 (1H, d, $J = 2.1$), 7.52 (1H, d, $J = 2.1$), 7.5 (2H, t, $J = 7.9$), 1.75 (2H, m), 1.10 (3H, t, $J = 7.7$), 4.85 (2H, s), 3.80 (2H, q, $J = 6.8$), 1.30 (3H, t, $J = 8.2$).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 162.7 (C-1), 133.4 (C-2), 133.1 (C-3), 162.1 (C-4), 118.6 (C-4a), 130.2 (C-5), 149.4 (C-6), 137.4 (C-7), 156.1 (C-8), 128.1 (C-8a), 190.1 (C-9), 119.1 (C-9a), 189.1 (C-10), 140.1 (C-10a), 44.1 (C-1'), 29.9 (C-2'), 20.6 (C-3'), 67.3 (C-1''), 72.5 (C-2''), 19.4 (C-3'').

3.6. Characterisation of compound 2

Brown gummy solid: UV $\lambda_{\text{max}}$ at 472 (3.0), 274 (3.3), 235 (5.4) nm. IR $v_{\text{max}}$ (KBr): 3390, 3406, 2910, 1696 cm$^{-1}$. EI-MS $m/z$: (rel. int.) 326 [M]+(99), 283.8 (35), 298 (29), 295.0 (21), 291.0 (23). HR-EI-MS: $m/z$ [M]+ calculated for C$_{19}$H$_{18}$O$_5$ 326.1154, found 326.1159.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.20 (1H, d, $J = 9.4$), 7.16 (1H, d, $J = 9.4$), 8.10 (1H, d, $J = 2.1$), 7.58 (1H, d, $J = 2.1$), 3.18 (2H, t, $J = 8.4$), 1.68 (2H, m), 1.52 (2H, m), 1.12 (3H, t, $J = 7.6$), 5.18 (2H, brs).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 160.1 (C-1), 131.5 (C-2), 130.9 (C-3), 160.7 (C-4), 118.7 (C-4a), 123.2 (C-5), 151.4 (C-6), 136.5 (C-7), 155.1 (C-8), 125.4 (C-8a), 192.1 (C-9), 118.1 (C-9a), 191.5 (C-10), 142.5 (C-10a), 39.1 (C-1''), 36.4 (C-2''), 28.1 (C-3''), 19.3 (C-4''), 61.3 (C-1'').

3.7. Characterisation of compound 3

Gummy solid: UV $\lambda_{\text{max}}$ at 474 (2.7), 270 (3.1), 234 (5.2) nm. IR $v_{\text{max}}$ (KBr): 3480 br, 1682, 2975 cm$^{-1}$. EI-MS $m/z$: (rel. int.) 312 [M]+(100), 280.9 (31), 269.7 (27), 276.9 (23). HR-EI-MS: $m/z$ [M]+ calculated for C$_{18}$H$_{16}$O$_5$ 312.0998, found 312.0105.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.15 (1H, d, $J = 10.1$), 7.10 (1H, d, $J = 10.1$), 8.01 (1H, d, $J = 1.9$), 7.48 (1H, d, $J = 1.9$), 3.21 (2H, t, $J = 8.2$), 1.82 (2H, m), 1.18 (3H, t, $J = 8.1$), 5.10 (2H, brs).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 161.2 (C-1), 133.4 (C-2), 132.6 (C-3), 160.1 (C-4), 119.8 (C-4a), 125.1 (C-5), 150.4 (C-6), 137.1 (C-7), 156.1 (C-8), 127.1 (C-8a), 190.7 (C-9), 120.1 (C-9a), 190.1 (C-10), 140.1 (C-10a), 44.1 (C-1''), 29.1 (C-2''), 20.1 (C-3''), 62.3 (C-1'').

4. Conclusion

The present study resulted in the isolation of anthraquinone analogues namely, 1,4-dihydroxy-6-(ethoxymethyl)-8-propylanthracene-9,10-dione (1), 1,4-dihydroxy-6-(hydroxymethyl)-8-butyl anthracene-9,10-dione (2) and 1,4-dihydroxy-6-(hydroxymethyl)-8-propyl anthracene-9,10-dione (3) from S. racemosa and characterisation was carried out using different spectroscopic techniques, i.e. 1D and 2D NMR, UV and IR as well as mass spectrometry.

Supplementary material

Supplementary material for this article is available online, along with NMR tables and structures of all compounds.

Disclosure statement

No potential conflict of interest was reported by the authors.
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