Dunaliene A, a new amino diketone from Desmos dunalii (Annonaceae)

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Dunaliene A (1), a new amino diketone, has been isolated from the leaves of Desmos dunalii together with four known dihydrochalcones: 2',4-dihydroxy-4',6'-dimethoxy-3',5'-dimethylchalcone (2), 2',4-dihydroxy-4',6'-dimethoxydihydrochalcone (3), 2',4-dihydroxy-4',5',6'-trimethoxydihydrochalcone (4) and 2',4-dihydroxy-5'-methyl-4',6'-dimethoxydihydrochalcone (5). The structures of these compounds were established notably by spectral analysis (1D- and 2D- ¹H, ¹³C NMR), UV, IR and HRMS.

Keywords: annonaceae; Desmos dunalii; dihydrochalcone; NMR

1. Introduction

The genus Desmos is widely distributed in India, Ceylon, Myanmar, Thailand, Indochina, the Philippines and Malaysia (Sinclair, 1955). In Malaysia, Burkill (1935) reported the use of Desmos by the native tribes, as postpartum medicine and to treat dysentery or vertigo. It is also mentioned that the leaves of some Desmos species were given to women after childbirth to increase the secretion of milk (Burkill, 1935).

Desmos species are known to be a rich source of bioactive alkaloids, flavonoids and aromatic compounds (Chan & Toh, 1986; Connolly, Dagli, & Haque, 2003; Shi, Pan, & Min, 2003; Sulaiman, Hadi, & Awang, 2003; Sulaiman, Martin, Pais, Hadi, & Awang, 1998; Sun et al., 1995; Wu et al., 2002). Desmosdumotin D, a chalcone with anti-HIV activity, was isolated from Desmos dumosus (Wu, Shi, Pang, Wang, & Yi, 2005). In addition, Nakagawa-Goto and Kuo-Hsiung (2006) isolated flavones possessing potent anti-tumour and anti-HIV properties. Desmos chinensis has been reported to produce the hydroxylated flavanone, 8-formyl-2,5,7-trihydroxy-6-methylflavone, which is a tyrosine kinase inhibitor (Kakeya et al., 1993). Previous chemical investigation on Desmos dunalii has produced four dihydrochalcones: 2',4-dihydroxy-4',5'-dimethoxy-3',6'-dimethylchalcone (2), 2',4-dihydroxy-4',6'-dimethoxydihydrochalcone (3), 2',4-dihydroxy-4',5',6'-trimethoxydihydro chalcone (4) and 2',4-dihydroxy-5'-methyl-4',6'-dimethoxydihydrochalcone (5) (Abdullah & Awang, 2005).

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Our continuing interest in this plant has led to the isolation of a new amino diketone derivative I. We herein report the isolation and structural elucidation of I and the pathway of its formation is also proposed. In addition, we also report the revised structure of 2',4-dihydroxy-4',5'-dimethoxy-3',6'-dimethylidihydrochalcone (Abdullah & Awang, 2005) to 2',4-dihydroxy-4',6'-dimethoxy-3',5'-dimethylidihydrochalcone (2).

2. Results and discussion

Dunaline A (1), \([\alpha]_D^{25} = -138^\circ (0.01, \text{MeOH})\), was isolated from the crude alkaloidal extract. It was afforded as a yellowish amorphous solid. The HREIMS showed a pseudomolecular ion peak at \(m/z\ 354.1304 \ [M + Na]^+\) (Calcd 354.1317, \(\Delta 1.3 \text{mmu}\)), corresponding to the molecular formula of \(\text{C}_{16}\text{H}_{21}\text{NO}_5\).

The \(^1\text{H NMR}\) spectrum indicated the presence of a para-substituted ring A pattern by revealing a pair of doublets representing two protons each at \(\delta 7.12\) and \(6.67 (J = 8.06 \text{ Hz})\), which are assignable to H-2/H-6 and H-3/H-5, respectively. The usual aromatic proton and carbon signals of ring B in dihydrochalcones were absent. Instead, the \(^{13}\text{C NMR}\) spectrum showed two carbonyl peaks at \(\delta 190.6\) (C6') and 199.3 (C2'), an oxygenated quaternary sp\(^3\) carbon signal at \(\delta 75.9\) (C3'), three sp\(^2\) carbons: \(\delta 104.0\) (C1'), \(\delta 119.1\) (C5'), one carbon bearing a methoxyl group at \(\delta 167.8\) (C4'), and two methyls at \(\delta 28.7\) and 9.7. The connectivities among C1', C2', C3', C4', C5' and C6' of ring B were established through HMBC correlations, as shown in Figure 1.

Two sets of multiplets centred at \(\delta 3.00\) and 2.70 were assignable to H2-\(\alpha\) and H2-\(\beta\), respectively. Dunaline A (1) is devoid of the carbonyl group usually found in dihydrochalcones since the signal at \(\sim \delta 205\) is lacking in the \(^{13}\text{C NMR}\) spectrum.

Figure 1. The \(^1\text{H} - ^1\text{H COSY}, \text{HMBC and NOESY correlations of 1 and 2.}\)
Interestingly, NH$_2$ proton signals appeared at $\delta$ 9.15 and $\delta$ 11.65, respectively, which suggested that the oxygen of the carbonyl may be replaced by an amino group.

The different resonances of both NH$_2$ protons indicated that one of them is most probably hydrogen bonded with the neighbouring carbonyl group (C2'). HMBC correlations were observed between H$\alpha$ C1', H$\alpha$ C1, H$\beta$/NH$_2$, H$\beta$/C2 and H$\beta$/C6, thus confirming that ring A and ring B are connected through (=C=NH$\cdot$-CH$_2$$\alpha$-CH$_2$$\beta$-). The assignments of all the carbons and protons of dunaliline A (1) were confirmed by thorough analysis of COSY, HMBC, HMQC and NOESY spectra.

Dunaliline A (1) is most likely an artefact arising from the reaction of 2',4-dihydroxy-3',5'-dimethyl-4',6'-dimethoxydihydrochalcone (2) with ammonia followed by oxidation. The proposed pathway of the formation of (1) is illustrated in Scheme 1 (Altun, Guallar, Friesner, Shaik, & Thiel, 2006; Derat & Shaik, 2006; Guengerich, 2003).

Compound 2, 2',4-dihydroxy-3',5'-dimethyl-4',6'-dimethoxydihydrochalcone, was isolated as an amorphous solid mass. The HREIMS exhibited a peak of [M + Na]$^+$ at m/z 353.1361 (Calcd 353.1365, $\Delta$0.4 mmu), consistent with the molecular formula of C$_{16}$H$_{12}$O$_5$. The structure of 2 was reported previously (Abdullah & Awang, 2005); however, in this communication the elucidation of the revised structure is briefly discussed. A reanalysis of the NOESY spectrum and the spectral data (H-$^1$H COSY, HMQC) of 2 has led to the structure of 2,4-dihydroxy-3',5'-dimethyl-4',6'-dimethoxydihydrochalcone. This revision was based on the fact that biogenetically, the C6' position is always oxygenated, rather than in position C5' (Whiting, 2001). To confirm this hypothesis, we have performed a NOESY experiment on 2. Evidently, the spectrum showed a correlation of 6'-OMe with the methylene protons of C$\alpha$, thus confirming the position of the methoxy at C6'.

![Scheme 1](image)
Table 1. $^1$H and $^{13}$C NMR (400 and 100 MHz) data of dundeline A (1) and 2',4-dihydroxy-4',6-dimethoxy-3',5-dimethylidihydrochalcone (2).

<table>
<thead>
<tr>
<th>Position number</th>
<th>$^1$H (δ) (J in Hz) a</th>
<th>$^{13}$C (δ) a</th>
<th>$^1$H (δ) (J in Hz) b</th>
<th>$^{13}$C (δ) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>104.0</td>
<td>-</td>
<td>111.5</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>199.3</td>
<td>-</td>
<td>160.9</td>
</tr>
<tr>
<td>3'</td>
<td>5.30 (OH) broad s</td>
<td>75.9</td>
<td>13.04, s (OH)</td>
<td>115.6</td>
</tr>
<tr>
<td>4'</td>
<td>-</td>
<td>167.8</td>
<td>-</td>
<td>163.4</td>
</tr>
<tr>
<td>5'</td>
<td>-</td>
<td>119.1</td>
<td>-</td>
<td>115.4</td>
</tr>
<tr>
<td>6'</td>
<td>-</td>
<td>190.6</td>
<td>-</td>
<td>158.8</td>
</tr>
<tr>
<td>C-NH$_2$</td>
<td>11.65, NH$_2$, broad s</td>
<td>178.6</td>
<td>-</td>
<td>206.3</td>
</tr>
<tr>
<td></td>
<td>9.10, NH$_2$, broad s</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C=O</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>3.00, 2H, m</td>
<td>40.1</td>
<td>3.37, 2H, t</td>
<td>44.9</td>
</tr>
<tr>
<td>β</td>
<td>2.70, 2H, m</td>
<td>35.3</td>
<td>2.93, 2H, t</td>
<td>29.3</td>
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<tr>
<td>1</td>
<td>-</td>
<td>133.2</td>
<td>-</td>
<td>133.2</td>
</tr>
<tr>
<td>2,6</td>
<td>7.12, 2H, d (8.06)</td>
<td>130.7</td>
<td>7.07, 2H, d (8.1 Hz)</td>
<td>129.5</td>
</tr>
<tr>
<td>3,5</td>
<td>6.67, 2H, d (8.06)</td>
<td>116.4</td>
<td>6.74, 2H, d (8.1 Hz)</td>
<td>115.3</td>
</tr>
<tr>
<td>4</td>
<td>9.15 (OH), sharp s</td>
<td>156.9</td>
<td>-</td>
<td>154</td>
</tr>
<tr>
<td>4'-OCH$_3$</td>
<td>3.90, 3H, s</td>
<td>61.9</td>
<td>3.72, 3H, s</td>
<td>60.0</td>
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<tr>
<td>6'-OCH$_3$</td>
<td>-</td>
<td></td>
<td>3.66, 3H, s</td>
<td>61.7</td>
</tr>
<tr>
<td>3'-CH$_3$</td>
<td>1.40, 3H, s</td>
<td>28.7</td>
<td>2.12 s</td>
<td>9.1</td>
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<tr>
<td>5'-CH$_3$</td>
<td>1.70, 3H, s</td>
<td>9.7</td>
<td>2.12 s</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Notes: $^1$H NMR in DMSO; $^{13}$C NMR in CD$_3$OD + one drop TFA; $^1$H- and $^{13}$C NMR in CDC$_3$.

instead of C5'. The revised $^1$H and $^{13}$C NMR data of 2 is listed in Table 1. The $^1$H-$^1$H COSY, HMBC and NOESY correlations of 2 are given in Figure 1.

The genus Desmos has produced many flavones and chalcones; however, the occurrence of dihydrochalcone is rather rare (Chan & Toh, 1986; Connolly et al., 2003; Shi et al., 2003; Sulaiman et al., 1998, 2003; Sun et al., 1995; Williams & Gray, 2004; Wu et al., 2002). This is the second report on this group in the Desmos species. The first Desmos plant reported to contain dihydrochalcones was D. chinensis (Kiem et al., 2003).

3. Experimental

3.1. General experimental procedure

Ultraviolet (UV) absorption spectra were recorded on a Shimadzu UV-160A UV-Visible spectrometer with methanol as a solvent. The IR spectra were obtained with CHCl$_3$ on a Perkin Elmer 1600 Double-Beam recording spectrometer. The optical rotations were recorded on Jasco (Japan) P1010 with tungsten lamp (concentration 0.01 w/v%). HRMS was obtained on Automet Multi Thermosyhnigan. 1D- and 2D-NMR analysis was carried out using FT-JEOL JMN-FX 100 (400 MHz), determined in CDCl$_3$, CD$_3$OD or DMSO-D$_6$. Column chromatography was performed using silica gel 60 F$_254$ in glass columns compressed with nitrogen gas. Aluminium supported silica gel 60 F$_254$ plates were used for thin layer chromatography (TLC). TLC spots were visualised under UV light.
(254 and 365 nm) followed by spraying with Dragendorff's reagent for alkaloid detection. Meyer's reagent was used for alkaloid screening.

3.2. Plant materials

The leaves of *D. dunali* were collected from the Segari Reserve Forest, Perak, Malaysia, in December 1996. A voucher specimen (KL.4668) was deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia, and also at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

3.3. Extraction and isolation of the alkaloids

A total of 1.6 kg of dried and grounded leaves of *D. dunali* was extracted with CH$_2$Cl$_2$. The extract obtained was acidified with 5% HCl solution. The organic (CH$_2$Cl$_2$) layer was dried under vacuum to give extract A (104 g). The acidic layer was subjected to the usual procedure for alkaloid extraction (Mukhtar et al., 2004) to give 3.6 g of crude alkaloid extract (Extract B).

Extract A underwent column chromatography over silica gel using CH$_2$Cl$_2$ with increasing proportions of MeOH to yield four neutral compounds: 2 (41.9 mg, CH$_2$Cl$_2$: MeOH, 99:1), 3 (157.5 mg, CH$_2$Cl$_2$: MeOH, 99:1), 4 (29.5 mg, CH$_2$Cl$_2$: MeOH, 95:5) and 5 (88.9 mg, CH$_2$Cl$_2$: MeOH, 95:5).

Extract B was also subjected to column chromatography, and subsequent purification by TLC (silica gel 60F$_{254}$) yielded compound 1 (20 mg, CH$_2$Cl$_2$: MeOH, 95:5).

*Dunaliene A* (1): light-brown amorphous, $[\alpha]_{D}^{20} = -138$ (0.01, MeOH); UV (MeOH): 203 (4.17), 214 (3.99), 228 (4.12), 268 (3.77), 303 (4.09); HREIMS: 354.1304 [M$^+$ + Na] (Calcd mass 354.1317, $\Delta1.3$ mmu). $^1$H NMR and $^{13}$C NMR data in Table 1.
2',4-Dihydroxy-4',6'-dimethoxy-3',5'-dimethyldihydrochalcone (2): Amorphous, UV \( \lambda_{max} \) (log \( e \)) (MeOH): 204 (4.39), 222 (4.39), 245 SH (3.46), 277 (4.14), 309 sh (3.35), 338 (3.53); IR \( \nu_{max} \) cm\(^{-1}\): 3408.76, 3018 18 2939.49, 1614.99; HREIMS: \( m/z \) 353.1361 (Calcd mass 353.1363, \( \Delta 0.4 \) mnu). EIMS: \( m/z \) 330 [M]+, 209, 120, 166, 151, 107; \(^1\)H NMR and \(^{13}\)C NMR data in Table 1.

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References


