

## New Isoflavonoid from *Dipterix odorata*

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The isoflavonol 7,8,3'-trihydroxy-6,4'-dimethoxy-isoflavone (**1**) was isolated from the methanol extract of the bark of the trunk of *Dipterix odorata*. The structure was determined from spectral data and with aid of its acetylation to obtain **1a** as a strategy to help the assignments of the 2D NMR experiments.

**Key words:** *Dipterix odorata*, Isoflavonol, 7,8,3'-Trihydroxy-6,4'-dimethoxy-isoflavone

### Introduction

The genus *Dipterix* comprises 14 species according to Ducke [1]. One of the more important plants of this genus is *Dipterix odorata* because of the commercial value of the seeds which are rich in coumarin. From *D. odorata*, coumarinols [2–4], isoflavonols [5,6], triterpenoids, fatty acids [7] and cassane-diterpenoids [8,9] have been isolated. Recently, polymethoxy flavonoids have shown a wide range of pharmacological effects [10,11]. In this paper we describe a new isoflavonoid obtained from the methanol extract of bark of the trunk of *D. odorata*. The characterization was aided by acetylation of compound **1** leading to the three-acetylated isoflavonol **1a**. The structural elucidation of these compounds was based on IR and Mass spectrometry and mainly by the 2D NMR experiments as NOESY and gradient selected COSY, COSY-LR (involving  $^1\text{H}$ ,  $^1\text{H}$  long range couplings), HMQC and HMBC [12–15].

### Results and Discussion

The presence of flavonoids was detected in the TLC color test with  $\text{FeCl}_3$  (green) and magnesium-HCl (orange) on the methanol extract of ground dried bark of the trunk of *Dipterix odorata*. The initial volume of this extract was reduced to 1/3 leading to a dark solution from which a solid material precipitated out. Subsequent recrystallization of the solid with methanol-hexane (2:1) yielded colorless crystals (**1**). The IR analysis of **1** shows the presence of aromatic rings (1607 and 1509  $\text{cm}^{-1}$ ), hydroxyl

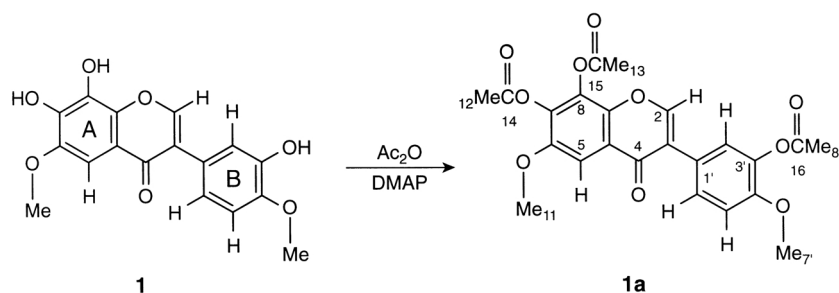
groups (3341  $\text{cm}^{-1}$ , very broad) and a conjugated carbonyl group (1665  $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum in  $\text{MeOH-d}_4$  reveals signals at  $\delta$  3.79 (s, 3H), 3.84 (s, 3H), 6.86 (d, 2H, 1.15 Hz), 6.95 (t, 1H, 1.15 Hz), 7.08 (s, 1H) and 8.07 (s, 1H). The high accuracy mass spectrum (ESI-TOF-MS) of this compound shows a molecular ion  $[\text{M}-\text{H}]^-$  with  $m/z$  329.0613 (calcd. 329.0661). Compound **1** has very low solubility, even in  $\text{MeOH-d}_4$  and thus needs considerable analysis time for accurate measurements of the  $^{13}\text{C}$  and DEPT NMR spectra. The following degrees of hydrogenation of each carbon were determined: two  $\text{CH}_3$  ( $\delta$  56.57 and 56.73), five CH ( $\delta$  96.49, 112.76, 117.58, 121.82 and 154.48) and ten non-hydrogenated carbons ( $\delta$  117.63, 125.17, 126.60, 134.84, 141.87, 144.64, 147.64, 148.70, 149.25, 178.17). All this data determined the molecular formula  $\text{C}_{17}\text{H}_{14}\text{O}_7$  for **1**, as well as the presence of two methoxyl groups, five aromatic hydrogens, two of which are coupled, and one conjugated carbonyl group. The chemical shifts of the carbon atoms in the  $^{13}\text{C}$  NMR spectrum together with the IR spectrum of **1** suggests the presence of three phenolic hydroxyl groups all of which determine a total of eleven degrees of unsaturation.

Compound **1** was acetylated with acetic anhydride in the presence of catalytic quantities of N,N-dimethylamino pyridine yielding a colorless compound **1a** (Scheme 1). The purpose of the acetylation was to improve the solubility, and thus to confirm the number of the phenolic hydroxyl groups and also to determine their positions. In addition, the acetylation induces certain changes in the  $^1\text{H}$  chemical shifts of

Table 1. Summary of the relationships observed in HMQC and HMBC experiments for compound **1a**.\*

Type	$\delta_C$	$H_{Ar}$ $\delta$ 7.90	$H_{Ar}$ $\delta$ 7.48	$H_{Ar}$ $\delta$ 7.24	$H_{Ar}$ $\delta$ 7.12	$H_{Ar}$ $\delta$ 6.91	OMe $\delta$ 3.79	OMe $\delta$ 3.72	CO <sub>2</sub> Me $\delta$ 2.27	CO <sub>2</sub> Me $\delta$ 2.22	CO <sub>2</sub> Me $\delta$ 2.17
CO <sub>2</sub> Me	19.85								$\Theta$		
CO <sub>2</sub> Me	19.89									$\Theta$	
CO <sub>2</sub> Me	20.28										$\Theta$
OMe	55.78							$\Theta$			
OMe	56.38						$\Theta$				
CH <sub>Ar</sub>	103.08		$\Theta$	+			+				
CH <sub>Ar</sub>	112.36					$\Theta$		+			
C <sub>Ar</sub>	122.29		++								
CH <sub>Ar</sub>	123.35			++	$\Theta$						
C <sub>Ar</sub>	123.75	+++			++						
C <sub>Ar</sub>	123.82				++	+++					
CH <sub>Ar</sub>	127.40			$\Theta$	++						
C <sub>Ar</sub>	132.77		+						+		
C <sub>Ar</sub>	137.73		+++							+	
C <sub>Ar</sub>	139.46			+	++	+++					+
C <sub>Ar</sub>	143.96	+++	+++								
C <sub>Ar</sub>	149.93		++				+++				
C <sub>Ar</sub>	151.23			+++	+++	+++		+++			
CH <sub>Ar</sub>	152.89	$\Theta$									
CO <sub>2</sub>	167.44									+++	
CO <sub>2</sub>	167.49								+++		
CO <sub>2</sub>	169.49										+++
CO <sub>Ar</sub>	175.31	+++	+++								

\* Solvent = CDCl<sub>3</sub>:MeOH-d<sub>4</sub> (4:1); cross signals seen in the HMQC spectrum =  $\Theta$ ; intensities of the cross signals seen in the HMBC spectrum: strong = +++, medium = ++, weak = +.



Scheme 1.

the two non equivalent hydrogens, which absorb at the same frequency in the <sup>1</sup>H NMR spectrum of **1**.

The mass spectrum (CG/EI-MS) of **1a** shows a molecular ion [M<sup>+</sup>] with *m/z* 456, that is consistent with the mass of compound **1** plus the mass of three acetyl groups confirming the presence of three phenolic hydroxyl groups on compound **1**. The <sup>1</sup>H NMR spectrum of **1a**, in CDCl<sub>3</sub>:MeOH-d<sub>4</sub> (4:1), shows the three methyls from the acetyl groups (CO<sub>2</sub>Me) at  $\delta$  2.17, 2.22, 2.27, and also two methoxyl groups (OMe,  $\delta$  3.72 and 3.79) and five aromatic hydrogens ( $\delta$  6.91, d, 8.48 Hz;  $\delta$  7.12, d, 2.13 Hz;  $\delta$  7.24, dd, 2.13 and 8.48 Hz;  $\delta$  7.48, s;  $\delta$  7.90, s). The <sup>1</sup>H,<sup>1</sup>H coupling patterns were obtained from the COSY ex-

periment, showing that three of the five aromatic hydrogens belong to a 1,3,4 trisubstituted ring (B-ring). The COSY-LR spectrum of **1a** shows correlations of the hydrogens of the methoxyl groups at  $\delta$  3.72 and 3.79 with the aromatic hydrogens at  $\delta$  6.91 and 7.48, respectively. This result shows that one of the methoxyl groups belongs to the B-ring. The hydrogenated carbons were assigned with aid of the HMQC (see Table 1 that summarizes the relationships).

The HMBC experiment shows the position of the three acetyl groups involving the C-7, C-8 and C-3' ( $\delta$  137.73, 132.77 and 139.46), due the weak cross signals of the methyl hydrogens 12, 13 and 8' ( $\delta$  2.22, 2.27 and 2.17) due to the <sup>4</sup>J<sub>CH</sub> couplings. These hy-

Carbon	Type	$\delta_{\text{C}}^{1\text{a}}$	$\delta_{\text{H}}^{1\text{a}}$	$\delta_{\text{C}}^1$	$\delta_{\text{H}}^1$
13	CO <sub>2</sub> Me	19.85	2.27 s	—	—
12	CO <sub>2</sub> Me	19.89	2.22 s	—	—
8'	CO <sub>2</sub> Me	20.28	2.17 s	—	—
7'	OMe	55.78	3.72 s	56.57	3.79 s
11	OMe	56.38	3.79 s	56.73	3.84 s
5	CH <sub>AR</sub>	103.08	7.48 s	96.49	7.08 s
5'	CH <sub>AR</sub>	112.36	6.91 d (8.48 Hz)	112.76	6.86 d (1.15 Hz)
10	C <sub>AR</sub>	122.29	—	117.63	—
2'	CH <sub>AR</sub>	123.35	7.12 d (2.13 Hz)	117.58	6.95 t (1.15 Hz)
3	C <sub>Ar</sub>	123.75	—	126.60	—
1'	C <sub>Ar</sub>	123.82	—	125.17	—
6'	CH <sub>AR</sub>	127.40	7.24 dd (2.13, 8.48 Hz)	121.82	6.86 d (1.15 Hz)
8	C <sub>Ar</sub>	132.77	—	141.87	—
7	C <sub>Ar</sub>	137.73	—	134.84	—
3'	C <sub>Ar</sub>	139.46	—	147.34	—
9	C <sub>Ar</sub>	143.96	—	144.64	—
6	C <sub>Ar</sub>	149.93	—	148.70	—
4'	C <sub>Ar</sub>	151.23	—	149.25	—
2	CH <sub>Ar</sub>	152.89	7.90 s	154.48	8.07 s
14	CO <sub>2</sub>	167.44	—	—	—
15	CO <sub>2</sub>	167.49	—	—	—
16	CO <sub>2</sub>	169.49	—	—	—
4	CO <sub>Ar</sub>	175.31	—	178.17	—

To reinforce the structure and stereochemistry of compound **1a**, we further carried out a NOESY experiment. The results are presented in Fig. 1, in which the principal  $^1\text{H}$ ,  $^1\text{H}$  NOE relationships observed are indicated by arrows. Note that the NOE observed between H-2 and H-13 is only possible for an isoflavonoid and not for a flavonoid skeleton. Table 2 summarizes the

Stems and pods were dried, ground, and treated with methanol at room temperature. The solvent was evaporated under vacuum. Column chromatography of the residue (hexane–EtOAc with increasing polarity), evaporation and recrystallization (methanol–hexane 2:1) yielded colorless crystals of **1** with m. p. 145–146 °C. Compound **1** was acetylated with acetic anhydride in the presence of DMAP in the usual manner, yielding colorless crystals of **1a** with m. p. 139–140 °C.

*7,8,3'-Trihydroxy-6,4'-dimethoxy-isoflavone (1)*

M.p. 145–146 °C. – IR (KBr):  $\tilde{\nu}$  = 3341, 1665 (C=O), 1607, 1509  $\text{cm}^{-1}$ . –  $^1\text{H}$  NMR (300.13 MHz, MeOH- $\text{d}_4$ ):  $\delta$  = 3.79 (s, 3H, OMe), 3.84 (s, 3H, OMe), 6.86 (d, 2H,  $J$  = 1.15 Hz, H-5' and H-6'), 6.95 (t, 1H,  $J$  = 1.15 Hz, H-2'), 7.08 (s, 1H, H-5) and 8.07 (s, 1H, H-2). –  $^{13}\text{C}\{^1\text{H}\}$  NMR (75.45 MHz, MeOH- $\text{d}_4$ ):  $\delta$  = 56.57 (OMe), 56.73 (OMe), 96.49 (C-5), 112.76 (C-5'), 117.58 (C-2'), 117.63 (C-10), 121.82 (C-6'), 125.17 (C-1'), 126.60 (C-3), 134.84 (C-7), 141.87 (C-8), 144.64 (C-9), 147.34 (C-3'), 148.70 (C-6), 149.25 (C-4'), 154.48 (C-2), 178.17 (C-4). – HRMS (ESI – / TOF):  $m/z$  (%) = exp. 329.0613 (100), calcd. 329.0661 [ $\text{M} - \text{H}$ ] $^-$ . – MS/MS (Argon, TOF):  $m/z$  (%) = 314.0434 (100) [ $\text{M} - \text{CH}_3 - \text{H}$ ] $^-$ , 299.0184 (50) [ $\text{M} - \text{CH}_3 - \text{H}$ ] $^-$ , 271.0348 (30) [ $\text{M} - \text{CO} - \text{H}$ ] $^-$ .

*7,8,3'-Triacetyl-6,4'-dimethoxy-isoflavone (1a)*

M.p. 139–140 °C. – IR (KBr):  $\tilde{\nu}$  = 1760 (C=O), 1666 (C=O), 1605, 1502  $\text{cm}^{-1}$ . –  $^1\text{H}$  NMR (300.13 MHz,

$\text{CDCl}_3$ :MeOH- $\text{d}_4$  4:1):  $\delta$  = 2.17 (s, 3H,  $\text{CO}_2\text{Me}$ ), 2.22 (s, 3H,  $\text{CO}_2\text{Me}$ ), 2.27 (s, 3H,  $\text{CO}_2\text{Me}$ ), 3.72 (s, 3H, OMe), 3.79 (s, 3H, OMe), 6.91 (d, 1H,  $J$  = 8.48 Hz, H-5'), 7.12 (d, 1H,  $J$  = 2.13 Hz, H-2'), 7.24 (dd, 1H,  $J$  = 2.13, 8.48 Hz, H-6'), 7.48 (s, 1H, H-5), 7.90 (s, 1H, H-2). –  $^{13}\text{C}\{^1\text{H}\}$  NMR (75.45 MHz,  $\text{CDCl}_3$ :MeOH- $\text{d}_4$  1:1):  $\delta$  = 19.85 ( $\text{CO}_2\text{Me}$ ), 19.89 ( $\text{CO}_2\text{Me}$ ), 20.28 ( $\text{CO}_2\text{Me}$ ), 55.78 (OMe), 56.38 (OMe), 103.08 (C-5), 112.36 (C-5'), 122.29 (C-10), 123.35 (C-2'), 123.75 (C-3), 123.82 (C-1'), 127.40 (C-6'), 132.77 (C-8), 137.73 (C-7), 139.46 (C-3'), 143.96 (C-9), 149.93 (C-6), 151.23 (C-4'), 152.89 (C-2), 167.44 (C-14), 167.49 (C-15), 169.49 (C-16), 175.31 (C-4). CG/EI-MS ( $\text{H}_2$ , EI 70 eV):  $m/z$  (%) = 456 (20) [ $\text{M}^+$ ].

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