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 his 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 cassanediterpenoids [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 ¹H, ¹H long range couplings), HMQC and HMBC [12-15].

Results and Discussion

The presence of flavonoids was detected in the TLC color test with FeCl₃ (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 recrystalization 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 cm⁻¹), hydroxyl

groups (3341 cm⁻¹, very broad) and a conjugated carbonyl group (1665 cm⁻¹). The ¹H NMR spectrum in MeOH-d₄ reveals signals at δ 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 $[M-H]^-$ with m/z 329.0613 (calcd. 329.0661). Compound 1 has very low solubility, even in MeOH-d₄ and thus needs considerable analysis time for accurate measurements of the ¹³C and DEPT NMR spectra. The following degrees of hydrogenation of each carbon were determined: two CH₃ $(\delta$ 56.57 and 56.73), five CH (δ 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 $C_{17}H_{14}O_7$ 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 ¹³C 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.

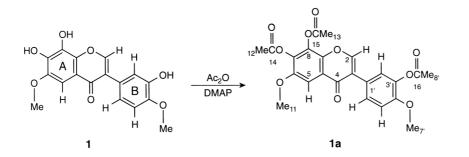
Compound 1 was acetylated with acetic anhydride in the presence of catalytic quantities of N,Ndimethylamino pyridine yielding a colorless compound 1a (Scheme 1). The purpose of the acetylation was to improve the solubility, and thus to confirm the number of the phenolic hydroxyl groups and also to determine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

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Туре	$\delta_{\rm C}$	H _{Ar}	OMe	OMe	CO ₂ Me	CO ₂ Me	CO ₂ Me				
		δ 7.90	δ 7.48	δ 7.24	δ 7.12	δ 6.91	δ 3.79	δ 3.72	δ 2.27	δ 2.22	δ 2.17
CO ₂ Me	19.85								Θ		
CO ₂ Me	19.89									Θ	
CO ₂ Me	20.28										Θ
OMe	55.78							Θ			
OMe	56.38						Θ				
CHAr	103.08		Θ	+			+				
CH _{Ar}	112.36					Θ		+			
CAr	122.29		++								
CH _{Ar}	123.35			++	Θ						
CAr	123.75	+++			++						
CAr	123.82				++	+++					
CH _{Ar}	127.40			Θ	++						
CAr	132.77		+						+		
CAr	137.73		+++							+	
CAr	139.46			+	++	+++					+
CAr	143.96	+++	+++								
CAr	149.93		++				+++				
CAr	151.23			+++	+++	+++		+++			
CHAr	152.89	Θ									
CO ₂	167.44									+++	
CO ₂	167.49								+++		
CO ₂	169.49										+++
COAr	175.31	+++	+++								

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

* Solvent = CDCl3:MeOH-d₄ (4:1); cross signals seen in the HMQC spectrum = Θ ; intensities of the cross signals seen in the HMBC spectrum: strong = +++, medium = ++, weak = +.



the two non equivalent hydrogens, which absorb at the same frequency in the ${}^{1}H$ NMR spectrum of **1**.

The mass spectrum (CG/EI-MS) of **1a** shows a molecular ion [M⁺] 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 ¹H NMR spectrum of **1a**, in CDCl₃:MeOH-d₄ (4:1), shows the three methyls from the acetyl groups (CO₂Me) at δ 2.17, 2.22, 2.27, and also two methoxyl groups (OMe, δ 3.72 and 3.79) and five aromatic hydrogens (δ 6.91, d, 8.48 Hz; δ 7.12, d, 2.13 Hz; δ 7.24, dd, 2.13 and 8.48 Hz; δ 7.48, s; δ 7.90, s). The ¹H,¹H 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 (<u>B</u>-ring). The COSY-LR spectrum of **1a** shows correlations of the hydrogens of the methoxyl groups at δ 3.72 and 3.79 with the aromatic hydrogens at δ 6.91 and 7.48, respectively. This result shows that one of the methoxyl groups belongs to the <u>B</u>-ring. The hydrogenated carbons were assigned with aid of the HMQC (see Table 1 that summarizes the relationships).

Scheme 1.

The HMBC experiment shows the position of the three acetyl groups involving the C-7, C-8 and C-3' (δ 137.73, 132.77 and 139.46), due the weak cross signals of the methyl hydrogens 12, 13 and 8' (δ 2.22, 2.27 and 2.17) due to the ${}^{4}J_{\rm CH}$ couplings. These hy-

Carbor	п Туре	$\delta_{\rm C}^{1a}$	$\delta_{ m H}{}^{ m 1a}$	$\delta_{\rm C}{}^1$	$\delta_{ m H}{}^1$
13	CO ₂ Me	19.85	2.27 s	-	
12	CO ₂ Me	19.89	2.22 s	-	
8'	CO_2Me	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	CHAR	103.08	7.48 s	96.49	7.08 s
5'	CHAR	112.36	6.91 d	112.76	6.86 d
			(8.48 Hz)		(1.15 Hz)
10	CAR	122.29		117.63	
2'	CHAR	123.35	7.12 d	117.58	6.95 t
			(2.13 Hz)		(1.15 Hz)
3	CAr	123.75		126.60	
1'	CAr	123.82		125.17	
6′	CHAR	127.40	7.24 dd	121.82	6.86 d
			(2.13, 8.48 Hz)		(1.15 Hz)
8	C _{Ar}	132.77		141.87	
7	CAr	137.73		134.84	
3′	CAr	139.46		147.34	
9	CAr	143.96		144.64	
6	CAr	149.93		148.70	
4'	CAr	151.23		149.25	
2	CHAr	152.89	7.90 s	154.48	8.07 s
14	CO_2	167.44		-	
15	CO_2	167.49		-	
16	CO_2	169.49		-	
4	CO _{Ar}	175.31		178.17	

Table 2. ¹H and ¹³C chemical shifts and ¹H, ¹H coupling constants from compounds **1** and **1a**.*

* J_{HH} in parenteses, solvent: MeOH-d₄ for **1** and CDCl₃:MeOH-d₄ (4:1) for **1a**.

drogens also show strong signals with the respective carbonyl carbons (δ 167.44, 167.49 and 169.49) due to ${}^{2}J_{CH}$. The location of the two methoxyl groups are defined by the cross signals between the methoxyl hydrogens at δ 3.72 and 3.79 (H-7' and H-11) with C-4' and C-6 (strong intensities due to ${}^{2}J_{CH}$ at δ 151.23 and 149.93) and with C-5' and C-5 (low intensities due to ${}^{4}J_{CH}$ at δ 112.36 and 103.08), respectively. This last assignment confirms the result of the COSY-LR above. The other ¹H, ¹³C long range relationships observed in the HMBC are described in Table 1. It should be noted that the two strong cross signals due to ${}^{3}J_{anti}$ couplings of the H-2 (δ 7.90) with C-9 and C-4 (δ 143.96 and 175.31), can only be assigned to an isoflavone structure for compound **1a** [12–15].

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 ¹H, ¹H 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

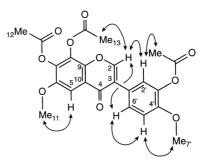


Fig. 1. Essential ¹H, ¹H NOE observed in the NOESY experiment of the compound **1a**.

hydrogen-1 and carbon-13 chemical shifts and the ¹H, ¹H coupling constants for compounds **1** and **1a**.

Experimental Section

General

The NMR experiments were carried out using a Bruker Avance DRX300 spectrometer (300.13 MHz for ¹H and 75.45 MHz for 13 C) with TMS as internal reference, equipped with an inverse multinuclear probehead and x,y,z gradient coils, and the pulse sequences and data processings controlled by the Xwin-nmr 1.3/9 software. The high accuracy MS data were obtained using negative ion electrospray ionization (ESI-TOF-MS) from 1:1 methanol:water solution (containing 2% of ammonium hydroxide) and the MS/MS analysis was obtained in the quadrupole selection from the [M-H]⁻ ion and 20 eV collision induced dissociation with argon in hexapole cell, in a Micromass QTOF spectrometer operating at 7000 mass resolution. The CG/EI-MS was done in a HP5987A spectrometer with quadrupole analyser (electron impact ionization, 70 eV) using H₂ as gas carrier in a glass capillary column (PS-090, $10 \text{ } m \times 0.3 \text{ } mm$). For CC silica gel 0.063-0.2 mm was used and for TLC, Kieselgel 60 (spots seen by UV 254 and 360 nm and I₂ vapor).

Plant material

Dipterix odorata was collected in Reserva Ducke in state of Amazonas, Manaus, Brazil, and identified at the Instituto de Botânica da Universidade de São Paulo (USP).

Extraction, isolation and acetylation

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 recrystalization (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): $\tilde{v} = 3341$, 1665 (C=O), 1607, 1509 cm⁻¹. – ¹H NMR (300.13 MHz, MeOH-d₄): $\delta = 3.79$ (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). – ¹³C{¹H} NMR (75.45 MHz, MeOH-d₄): $\delta = 56.57$ (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 [M-H]⁻. – MS/MS (Argon, TOF): m/z (%) = 314.0434 (100) [M - CH₃ - H]⁻, 299.0184 (50) [M - CH₃ - H]⁻, 271.0348 (30) [M - CO - H]⁻.

= H-6'), 7.48 (s, 1H, H-5), 7.90 (s, 1H, H-2). $-{}^{13}C{}^{1}H$ NMR (75.45 MHz, CDCl₃:MeOH-d₄ 1:1): δ = 19.85 (CO₂Me), 19.89 (CO₂Me), 20.28 (CO₂Me), 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 (H₂, EI 70 eV): *m*/*z* (%) = 456 (20) [M⁺].

CDCl₃:MeOH-d₄ 4:1): δ = 2.17 (s, 3H, CO₂Me), 2.22 (s, 3H, CO₂Me), 2.27 (s, 3H, CO₂Me), 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,

Acknowledgements

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 cm^{-1}. – 1H NMR (300.13 MHz,

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