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Chemical Constituents from *Caesalpinia férrea*: Identification and ^{1}H and ^{13}C Resonance Assignment

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Abstract

In a phytochemical investigation of *Caesalpinia ferrea* (Leguminosae), four aromatic compounds (1-4) have been isolated and identified. Their structures have been assigned based on data provided by spectroscopic techniques, including 2D NMR experiments. Compounds 3 and 4 are being reported for the first time for *Cesalpina ferrea.*

Keywords

***Caesalpinia ferrea*, Leguminosae, Aromatic Compounds, Spectral Studies**

1. Introduction

The genus *Caesalpinia* comprises ca. 100 species, distributed widely in tropical and subtropical regions [1]. About 17 species of the genus are widespread in China, and 14 species of the genus have long been used in Chinese traditional medicine for the treatment of rheumatism and inflammatory diseases [2]. *Caesalpinia ferrea* Mart. is a species belonging to Leguminosae family commonly known in Brazil as "jucá" or "pau-ferro". It occurs in Brazil from the Northeast Region to the State of Rio de Janeiro and it is widely utilized in folk medicine due to its several therapeutic properties such as anti-inflammatory, analgesic, antimicrobial and antipyretic [3]. From an ethanol extract of *Caesalpinia ferrea* four phenolic compounds were isolated and identified by spectral

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data: organic acid **1** and ester **2** (from green beans), biflavonoid **3** and phytoalexin **4** (from stem). Additionally, from this same extract, other constituents (two triterpenes, two steroids, two acid and fatty alcohol) were detected only by GC/MS. The structures of phenolic compounds were elucidated based on spectral studies, especially 1D and 2D NMR experiments.

2. Results and Discussion

Compound **1** was isolated as a white amorphous solid from EtOH extract of the green beans of *C. ferrea* by Sephadex LH-20 column (MeOH). The IR spectrum showed bands at 3600 - 2500 cm^{-1} (broadband indicative of the OH group of carboxylic acid), 1689 cm^{-1} (carboxyl group) and 1610, 1542 and 1448 cm^{-1} (aromatic ring). ^1H NMR spectrum of **1** exhibited one only signal at δ_H 7.06 (s, 2 H) which indicates a tetra substituted aromatic ring containing two equivalent hydrogens. The ^{13}C NMR spectrum exhibited five resonances, however the signals at δ_C 145.52 and 110.53 with high relative intensities, were assigned to two carbon atoms each. Therefore, there appeared to be seven carbons in **1**. All the signals in the ^{13}C NMR spectrum were in the region of sp^2 carbons (included those in δ_C 170.52 assigned to carbonyl carbon of a conjugated acid carboxylic), and the comparison with the ^{13}C NMR DEPT spectrum showed four signals to five non-hydrogenate carbons and one signal to two methine carbons. The analysis of these spectral data (**Table 1**) and comparison with spectral data of literature [4] [5] identified **1** as 3,4,5-trimethoxybenzoic acid, commonly known as gallic acid (**Figure 1**).

Compound **2** was obtained as a yellow amorphous solid. The EtOH extract of the green beans was partitioned between hexane and EtOAc and fraction hexane was subjected to cc Si gel to yield **2**. ^1H NMR of **2** exhibited one only signal at δ_H 7.45 (s, 2 H) which indicates a substituted aromatic ring containing two equivalent hydrogens as in **1**. The ^{13}C NMR of **2** exhibited seven sp^2 carbons signals, and the comparison with the DEPT ^{13}C NMR spectrum showed one signal to methine (δ_C 110.25) and six signals to non-hydrogenated carbons (**Table 1**). The only record in the HMQC experiment on **2** correlated the carbon signal at δ_C 110.25 with the singlet of the two aromatic hydrogens at δ_H 7.45 (2 H), while the HMBC experiment correlated these two hydrogens with carbons signals at δ_C 107.61; 112.37; 139.71; 148.17 and 159.20. Thus, the structure of **2** was determined on the basis of 2D-NMR spectroscopy and by comparison with spectral data of literature [6] to be a derivative ester dimer from gallic acid, the 4,4',5,5',6,6'-hexahidroxydifenic-2,6,6'-dilactone, known as ellagic acid (**Figure 1**).

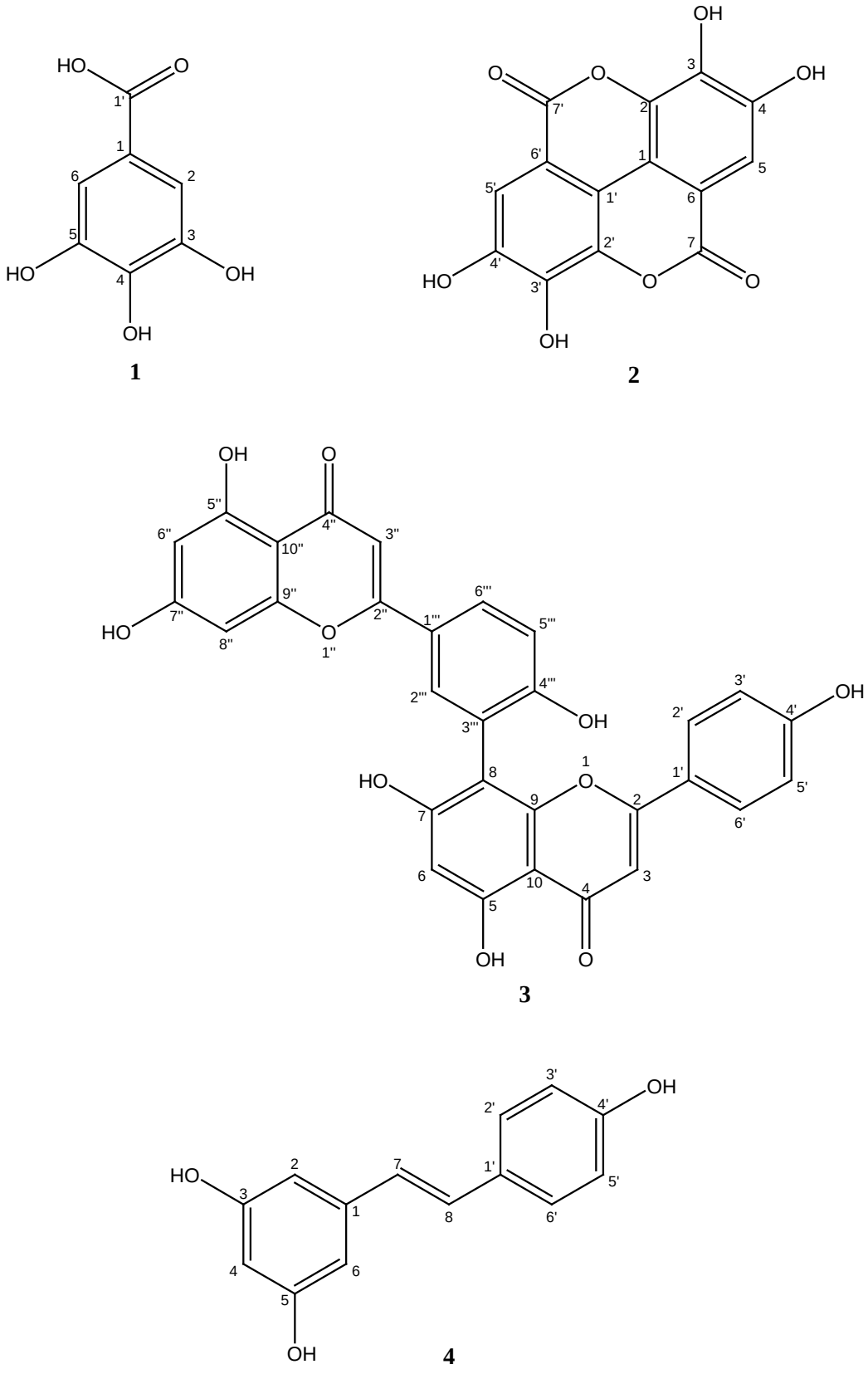
Compound **3** was obtained as a white amorphous solid from EtOH extract of the stem of *C. ferrea* by Sephadex LH-20 column (MeOH). The IR spectrum showed bands at 3100 cm^{-1} (broad band typical of one or more OH groups), 1618 cm^{-1} (chelated carbonyl or conjugated double bond), 1571, 1498 and 1450 cm^{-1} (aromatic ring), 1233, 1161 cm^{-1} (C-O/C-C bounds) and 820 cm^{-1} (=C-H bounds). The ^{13}C NMR spectrum (**Table 2**) showed 28 signals, all as sp^2 carbons, including two to carbon carbonyl atoms (δ_C 183.96 and 184.24). The comparison with the DEPT 135 NMR spectrum revealed 18 quaternary and 10 methines carbons. However, the methine carbon signals at δ_C 129.46 and 116.94 with high relative intensities, each correlated to two carbon atoms, then the spectra indicated the presence of 30 carbon atoms, consistent with the compound being a biflavonoide with molecular formula $C_{30}H_{18}O_{10}$ in accordance with the peaks at m/z 539 $[M + H]^+$ and 537 $[M - H]^-$ in the HR-TOF-MS spectra obtained using ESI ionization. In the ^1H NMR spectrum, an AA'BB' benzenoid spin system was inferred from the signals at δ_H 7.58 (d, 2 H, J = 8.7 Hz) and 6.67 (d, 2 H, J = 8.7 Hz) in accordance with the signals of methine carbons in δ_C 129.46 (2CH) and 116.94 (2CH). The ^1H NMR spectrum also exhibited two singlet of one hydrogen each at δ_H 6.61 and 6.59, characteristic of flavone units (hydrogens attached to the C-3 in the flavonoid skeleton); signals at δ_H 8.07 (1 H, d, J = 2.1 Hz), 7.89 (1 H, dd, J = 8.6, 2.1 Hz) and 7.10 (1 H, d, J = 8.6 Hz) revealed an AMX coupling system in the 3'''-4'''-bisubstituted $\mathbf{B_I}$ ring of **3** indicating that C-3''' was the position of linkage of the two flavonoids units [7]; two *meta*-coupled hydrogens signals in $\mathbf{A_I}$ ring appeared at δ_H 6.17 (1 H, d, J = 1.8 Hz) and 6.29 (1 H, d, J = 1.8 Hz). Thus, the signals of the hydrogen atoms of the flavonoid unit **I**, were: δ_H 6.61 (s, H-3"), 6.17 (d, H-6"), 6.29 (d, H-8"), 8.07 (d, H-2'''), 7.89 (dd, H-6''') and 7.10 (d, H-5'''). Further, one hydrogen signal appeared at δ_H 6.32 (1H, s) which was attributed to the hydrogen H-6 ($\mathbf{A_{II}}$ ring) assuming that C-8 was the position of linkage of the two flavonoid units. Thus, the signals of the hydrogen atoms of the flavonoid unit **II**, were: δ_H 6.59 (s, H-3), 6.32 (s, H-6), 7.58 (d, H-2'/H-6') and 6.67 (d, H-3'/H-5'). All the chemical shifts of carbons connected with hydrogens were confirmed using the HSQC experiment (**Table 2**). The HMBC spectrum showed that H-6 (δ_H 6.32) and H-2''' (8.07) were correlated with resonances at δ_C 106.91 (C-8) and that H-5''' (7.10) was correlated with the resonance at 122.59 (C-3'''). These correlations were important to confirm that the linkage between both flavonoid units occurred by $\mathbf{B_I}$ and

Table 1. ^{1}H and ^{13}C spectral data for compounds **1** and **2** in CD_3OD.

1			**2**			HMBC	
HSQC			HSQC				
C	δ_C	δ_H	**C**	δ_c	δ_H	$^{2}J_{C-H}$	$^{3}J_{C-H}$
1	122.25	-	1, 1'	107.61	-	-	H-5/H-5'
2/6	110.53	7.06 (s)	2, 2'	136.41	-	-	-
3/5	146.52	-	3, 3'	139.71	-	-	H-5/H-5'
4	139.70	-	4, 4'	148.17	-	H-5/H-5'	-
1'	170.59	-	5, 5'	110.25	7.46 (s)	-	-
			6, 6'	112.37	-	H-5/H-5'	-
			7, 7'	159.20	-	-	H-5/H-5'

Table 2. ^{1}H and ^{13}C spectral data for compound **3** in CD_3OD.

	HSQC		HMBC		^{1}H-^{1}H COSY
	δ_C	δ_H	$^{2}J_{C-H}$	$^{3}J_{C-H}$	
C					
2	166.56	-	H-3	H-2'/H-6'	
3	103.41	6.59 (s)			
4	183.96	-	H-3		
5	162.38	-	H-6		
6	101.64	6.32 (s)			
7	162.55	-	H-6		
8	106.91	-		H-2'", H-6	
9	156.70	-			
10	104.93	-		H-3, H-6	
1'	123.55	-		H-3'/H-5'	
2'/6'	129.46	7.58 (d, 8.7 Hz)			H-3'/H-5'
3'/5'	116.95	6.67 (d, 8.7 Hz)			H-2'/H-6'
4'	163.28	-		H-2'/H-6'	
2"	167.25	-	H-3"	H-2'"	
3"	103.93	6.61 (s)			
4"	184.24	-	H-3"		
5"	165.92	-	H-6"		
6"	100.36	6.17 (d, 1.8 Hz)		H-8"	H-8"
7"	166.30	-			
8"	95.29	6.29 (d, 1.8 Hz)		H-6"	H-6"
9"	159.52	-	H-8"		
10"	105.37	-		H-3", H-6", H-8"	
1'"	123.41	-		H-5'"	
2'"	132.96	8.07 (d, 2.1 Hz)		H-6'"	H-6'"
3'"	122.59	-		H-5'"	
4'"	162.67	-		H-2'"/H-6'"	
5'"	118.97	7.10 (d, 8.6 Hz)			H-6'"
6'"	128.56	7.89 (dd, 8.6; 2.1 Hz)		H-2'"	H-2'"; H-5'"



1 **2** **3** **4**

Figure 1. Phenolic compounds isolated from *C. ferrea*.

A_{II} rings, and indicated **3** as a biflavonoid (**Figure 1**) with a C-8-C-3''' interflavonoid linkage corresponding to the amentoflavone series [7] [8]. The ^{1}H- and ^{13}C NMR signal assignments (**Table 3**) were achieved by combination of ^{1}H-H COSY, HSQC and HMBC spectral data, and comparison with literature values [9]. To our knowledge, this is the first report of isolation of **3** from *Caesalpinia ferrea.*

Compound **4** was obtained as a yellow amorphous solid from EtOH extract of the stem of *C. ferrea* by Sephadex LH-20 column (MeOH). The IR spectrum of **4** showed intense broadband at 3204 (OH groups), 1584, 1509 and 1460 (aromatic ring), 1146, 986, 963 (C-O/C-C bounds) and 827 cm^{-1} (=C-H bounds). Analysis of the NMR spectral data showed that **4** was composed of two substituted phenyl rings connected by one double bond. The two olefinic hydrogens [δ_H 6.79 (1 H, d, J = 16.0 Hz) and 6.95 (1 H, d, J = 16.0 Hz)] showed a $^{3}J_{HH}$ = 16.0 Hz indicating a trans-configuration. Further, examination of the ^{1}H NMR spectrum indicated the presence of one symmetrically trisubstituted aromatic ring [δ_H 6.15 (1 H, t, J = 2.1 Hz) and 6.44 (2 H, d, J = 2.1 Hz)], and one 1,4-disubstituted aromatic ring [δ_H 6.76 (2 H, d, J = 8.6 Hz) and 7.34 [2 H, d, J = 8.6 Hz)]. The ^{13}C NMR spectrum displayed ten signals, six methine and four quaternary carbon atoms; chemical shift suggested that tree carbons were oxygenated (δ_C 159.80 (2C) and 158.51 (C)), and thus consistent with the structure of *trans*-3,5,4'-trihydroxyestilbene (**4**), as supported by the HMBC spectrum through the correlations of H-2/H-6 (δ_H 6.44) with C-3/C-5 (δ_C 159.80), H-2'/H-6' (δ_H 7.34) and H-3'/H-5' (δ_H 6.76) with C-4' (δ_C 158.51). **Table 3** gives the ^{1}H and ^{13}C NMR chemical shift assignments of **4** and have been confirmed by DEPT, ^{1}H-^{1}H COSY, HSQC and HMBC experiments. In addition, spectral data of compound 4 were compared with spectral data of literature [10]. To our knowledge, this is the first report of isolation of **4**, known as resveratrol (**Figure 1**), from *Caesalpinia ferrea.*

3. Experimental

3.1. General

IR spectrum were recorded on a Perkin-Elmer model Spectrum 100 FTIR spectrophotometer using KBr disks. NMR data were performed on Bruker DPX 300 and DRX 500 spectrometers, with TMS as internal standard. Mass spectra were determined on Shimadzu QP 5050A spectrometer, and HR-ESI-MS were acquired using a Q-TOF mass spectrometer. Column chromatography (CC) was conducted using silica gel 60 (0.040 - 0.0063 mm; 230 - 400 mesh, Merck), and TLC was performed on precoated silica gel polyester sheets (kieselgel 60 F_{254}, 020 mm, Merck). All compounds were detected by spraying with vanlin/perchloric acid/EtOH solution followed by heating at 100˚C.

3.2. Plant Material

The pods and stems of *C. ferrea* were collected at Acarape County, State of Ceará, Brazil. A voucher sample is deposited in the Herbarium Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará.

Table 3. ^{1}H and ^{13}C spectral data for compound **4** in CD_3OD.

	HSQC		HMBC		^{1}H-^{1}H COSY
	δ_C	δ_H	$^{2}J_{C-H}$	$^{3}J_{C-H}$	
C					
1	141.47	-		H-8	
2/6	105.96	6.45 (d, 2.2 Hz)		H-6/H-7	H-4
3/5	159.80	-	H-2		
4	102.84	6.16 (t, 2.0 Hz)	H-2/H-6		H-2/H-6
7	127.20	6.79 (d, 16.2 Hz)		H-2/H-6	H-8
8	129.56	6.96 (d, 16.0 Hz)	H-7		H-7
1'	130.61	-		H-3'/H-5'	
2'/6'	128.93	7.35 (d, 8.6 Hz)		H-8/H-6'	H-3'/H-5'
3'/5'	116.65	6.76 (d, 8.6 Hz)			H-2'/H-6'
4'	158.51	-	H-3'/H-5'		

3.3. Extraction and Isolation

Dried and powdered pods (530.0 g) were extracted successively with hexane and EtOH at room temperature. The extracts were concentrated under vacuum to yield 1.5 and 121.2 g, respectively. The crude EtOH extract (70.5 g) was suspended in a MeOH:H_2O (3:7 v/v) and partitioned with hexane, CH_2Cl_2, EtOAc and MeOH. The EtOAc fraction (500.1 mg) was then subjected to Sephadex LH-20 column (MeOH) to give 127 fractions. The fractions were monitored by TLC and fraction 23 - 25 yielded compound **1** (25.0 mg) as orange crystals. The hexane fraction (200.1 mg) was subjected to silica gel column chromatograpy eluted with hexane:EtOAc 1:1, EtOAc, Acetone and MeOH. Fraction acetone afforded compound **2** (12.1 mg) as amorphous yellow solid. Dried and powdered stem (4.4 kg) were extracted successively with hexane and EtOH at room temperature. The extracts were concentrated under vacuum to yield 20.3 and 32.6 g, respectively. The crude EtOH extract (32.6 g) was suspended in a MeOH:H_2O (3:7 v/v) and partitioned with hexane, CH_2Cl_2 and EtOAc. The solutions were dried ($MgSO_4$) and concentrated under reduced pressure. The fraction CH_2Cl_2 (2.0 g) was then subjected to Sephadex LH-20 column (MeOH) to give 5 fractions coded as CEM-20. The fraction CEM-20-4 (64.5 mg) was further subjected to Sephadex LH-20 column (MeOH) to yield 21 fractions. These fractions were monitored by TLC and fraction 18 - 21 (25.0 mg) was subjected to Si gel cc eluted with hexane-CH_2Cl_2 8:2, CH_2Cl_2, CH_2Cl_2-EtOAc (9:1, 8:2, 6:4 and 4:6) and EtOAc to give 17 fractions. These fractions were monitored by TLC and fractions 14 - 17 (EtOAc) afforded **3** (20.0 mg). The fraction CEM-20-2 (144.0 mg) was further subjected to Sephadex LH-20 column (MeOH) to yield 24 fractions. These fractions were monitored by TLC and fraction 22 - 24 afforded compound **4** (15 mg) as a yellow solid.

4. Conclusion

This work demonstrated a practical application of spectroscopic techniques in the identification of natural products. Thus, using high-resolution mass spectrometry, and especially the two-dimensional NMR spectroscopy, two previously unpublished components (**3** and **4**) were characterized from the species *Caesalpinia ferrea*, which are of potential importance to human health as antioxidant and also in food.

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