^{1}H and ^{13}C NMR Application to Structure Elucidation of Prenylated Naphthoquinone Dimers from *Lippia microphylla*.

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Abstract: *Two prenylated naphthoquinone dimers, a known and a new one, were isolated from roots of Lippia microphylla. Their structures were determined by spectroscopic methods (IR and MS) including detailed 1D and 2D NMR (BBHD, DEPT, HMQC, HMBC and NOESY) analysis. The unambiguous assignment of all NMR data with all hydrogens and carbons of the novel compound is provided.*

Resumo: *Duas naftoquinonas diméricas preniladas, uma conhecida e outra inédita, foram isoladas das raízes de Lippia microphylla. Suas estruturas foram determinadas por métodos espectroscópicos (IV and EM) incluindo detalhada análise de RMN 1D e 2D (BBHD, DEPT, HMQC, HMBC e NOESY). A correlação inequívoca de todos os dados de RMN com todos os átomos de hidrogênio e carbono da estrutura proposta para o novo composto é sugerida*

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Introduction

The *Lippia* genus (Verbenaceae) is a prolific source of flavouring plants, most of them with folk medicinal use. In the Northeast of Brazil two *Lippia* species are widely used: *L. sidoides*, popularly known as " alecrim-pimenta" of topical use as a general antiseptic,[1] and *L. alba*, popularly designated as "erva cidreira", whose leaves infusion is used as a soothing tea.[2]

A literature survey revealed several papers about Lippia species but no phytochemical report was found for L. microphylla, popularly known as "alecrim-de-tabuleiro". Despite not being currently used in popular medicinal it was chosen as the subject of the present work.

Experimental

Plant material

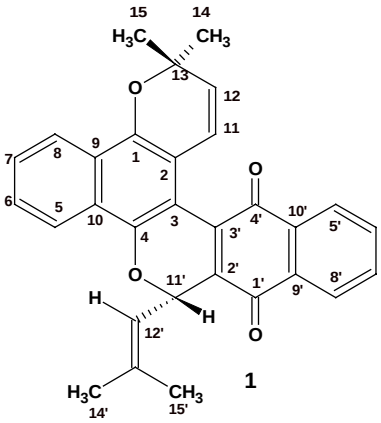
Roots of L. microphylla (Verbenaceae) were collected in April 1999, in Várzea Alegre, Ceará State – Brazil, and authenticated by Prof. Afrânio Gomes Fernandes of the Departamento de Biologia, Universidade Federal do Ceará. A voucher specimen (No 2287) has been deposited in the Herbarium Prisco Bezerra - UFC.

Extraction and isolation

The dried, ground biomass (940 g) from the roots of L. microphylla was extracted at room temperature with EtOH. After solvent evaporation under reduced pressure, a brown residue (30 g) was obtained. This material was fractioned over a Silica gel column with hexane/CHCl3 (1:1), CHCl3, EtOAc and MeOH. The hexane/CHCl3 (1:1) fraction was chromatographed over Silica gel by elution with 0–100% EtOAc-hexane mixtures. The residue from the 5% EtOAc-hexane fraction was subjected to repeated column chromatography to yield 1 (15 mg, m.p. 188 °C) and 2 (100 mg, m.p. 204 °C) as pure compounds.

NMR spectra

1H and 13C spectra were determined on a Bruker DRX 500 spectrometer at 27 °C operating at 500.13 MHz for 1H and 125.77 MHz for 13C. The samples [1 (15 mg) and 2 (50 mg)] were dissolved in 0.5 mL of CDCl3 or DMSO-d6 and set into 5.0 mm NMR tubes (Norell, Inc., #508-UP RB). Internal lock and residual CHCl3 (δ 1H =7.24) and 13CDCl3 (13C = 77.00) signals were used as references for 1 and 2.



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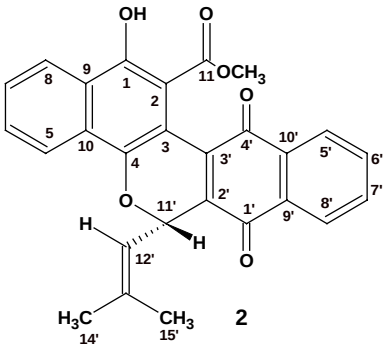

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The acquisition was performed by standard Bruker pulse programs [zg30 (^{1}H), zgpg30 (^{13}C-BBHD), dept135 (^{13}C-DEPT 135), cosy90 (^{1}H,^{1}H-COSY), invbtp (HMQC), inv4lplrnd (HMBC) using either a 5 mm dual $^{13}C/^{1}H$ probe for normal (^{13}C) detection or 5 mm multinuclear inverse Z-grad. probe for inverse (^{1}H) detection. 32 and 64K data point sets, with spectral width of 12 and 31 KHz,

The NOESY spectrum was obtained with 5 KHz spectral width for both F2 and F1. 1K x 1K data blocks, with phase adjustment for both F2 and F1, followed by final data matrix symmetrization were used for processing. Other parameters were: 256 increments in t1, 64 transients and relaxation delay of 1 s and a mixing time of 650 ms.

Results and Discussion

Silica gel column chromatography of the hexane/$CHCl_3$ fraction from the EtOH extract of roots of *L. microphylla*, using *n*-hexane with increasing concentrations of EtOAc as eluent, yielded the two naphthoquinones, 1 and 2.

Naphthoquinone **1**, was isolated as a green powder, m.p. 188 °C. Spectrometric analysis, particularly by one and two-dimensional NMR, allowed its identification as tecomaquinone I (**1**), by comparison to the data already reported in the literature.[3] Tecomaquinone I had been previously isolated from *L. sidoides*, a congener of *L. microphylla.*[3]

Naphthoquinone **2**, was isolated as colorless prisms, m.p. 204 °C. Its IR spectrum showed absorption bands for hydroxyl (3438 cm^{-1}) and were collected for ^{1}H and ^{13}C one-dimensional spectra, respectively. Inverse detected 2D heteronuclear correlated spectra were collected over 1K data points, with spectral width of 5 KHz and 256 points in F2, and 27 KHz in F1. Data processing were performed with 1K x 256w blocks, using backward linear prediction in F1 to generate the final data matrix. conjugated carbonyl groups (1660 cm^{-1}). The molecular ion peak with *m/z* 440 Daltons, in conjunction with the ^{1}H and BB spectra (Table 1) showing 27 signals, allowed the determination of its molecular formula as $C_{27}H_{20}O_6$. The DEPT 135° spectrum revealed three methyl groups, one of which was a methoxy group (δ 52.17), one methine, nine monohydrogenated sp^2 carbons, and by comparison to the BB spectrum, fourteen non-hydrogenated sp^2 carbons, including three carbonyl carbons (δ 184.83, 182.53 and 171.24) and two oxygenated carbons (δ 154.75 and 147.83). ^{1}H and ^{13}C NMR data comparison of both **1** and **2**, revealed a striking similarity, except for the missing cyclic isoprene moiety for **2**, compared to **1** and, in addition, the presence of a carbomethoxy group for **2**. This strongly supports the oxidation at C-11, generating the carbomethoxy group, and the consequent elimination of the remaining four carbons unity of that side chain in **1**. Even though a more deshielded absorption for C-3 (conjugated with the carbomethoxy) was expected, initially the complete ^{13}C data assignment was a difficult task because the absence of any hydrogen long-range correlation with C-3 or C-2 (this one is *ortho* to the

hydroxy, but is conjugated to the quinone C-1`) did not allow an unambiguous assignment of those carbons. Fortunately, the HMBC data obtained in dry DMSO-$d_6$ revealed long-range correlations of the hydroxy hydrogen (δ 10.88) with C-1 (δ 154.75), C-9 (δ 127.71) and C-2 (δ 104.43). In addition, the distinction between C-2`(δ 133.37) and C-3` (δ 137.41), both conjugated to the quinone carbonyls, was done by the correlations of the carbinolic like, and doubly allylic H-11`(δ 6.51) with C-4 (δ 142.83),

C-2` (δ 133.37), C-3` (δ 137.41) and C-1` (δ 182.53), as well as by the correlations of the vinyl hydrogen H-12` (δ 5.60) with C-14`(δ 26.40) and C-15`(δ 19.30).

Figure 1 shows some significant correlations observed through the HMBC experiment. Finally the relative stereochemistry suggested for **2**, named microphyllaquinone, similarly to **1**, was based on the NOESY analysis, Figure 2.

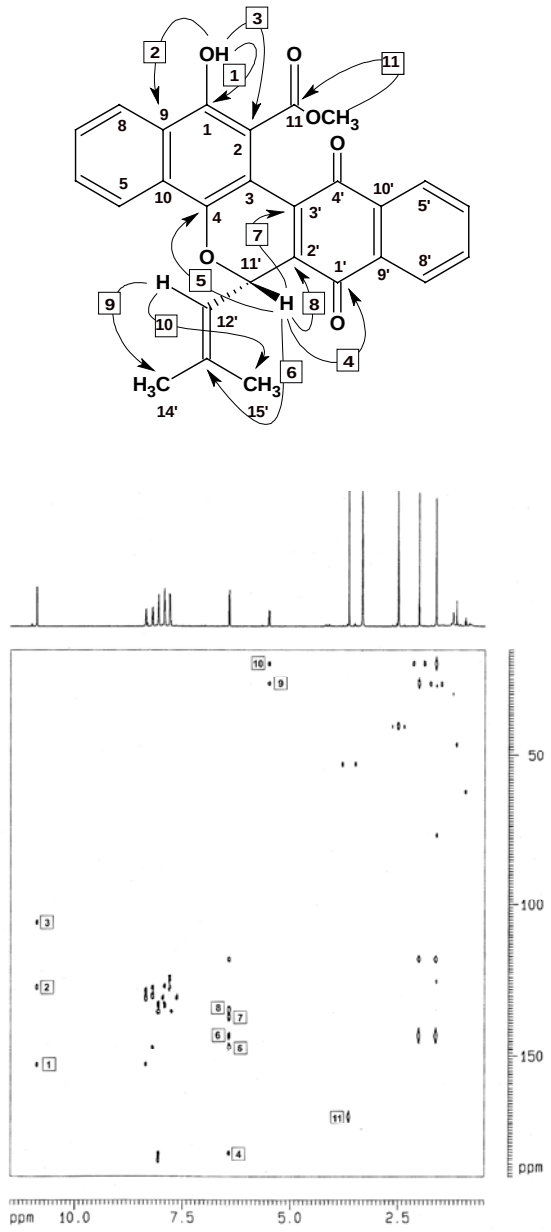

Figure 1. Interpreted HMBC spectrum of **2** (500/125 MHz, DMSO-d_6):numbered arrows indicate long-range heteronuclear C-H correlations whose signals are marked with the same number.

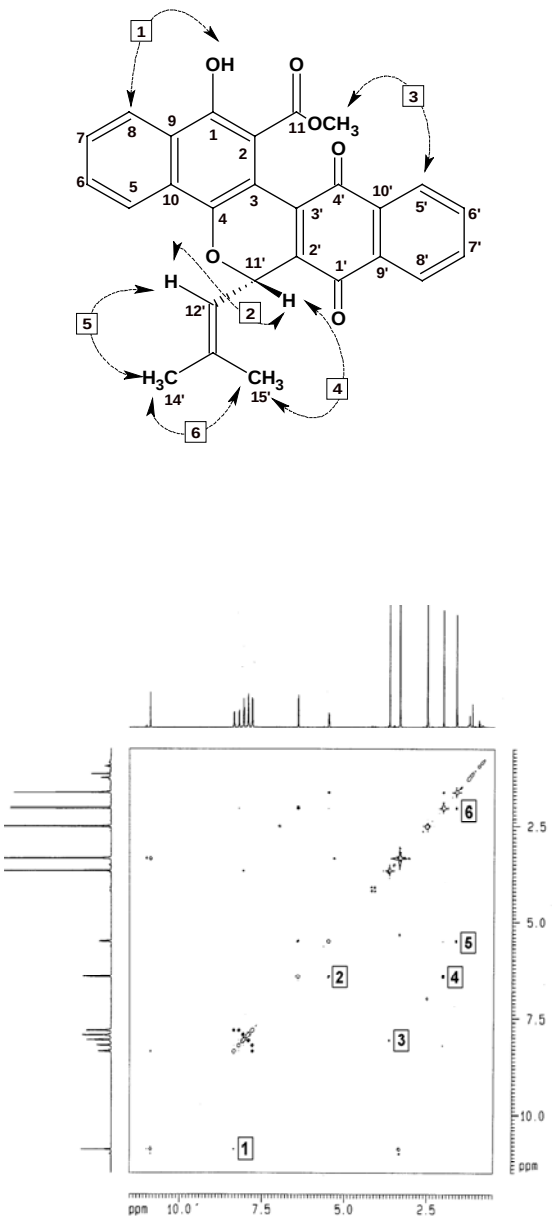

Figure 2. Interpreted NOESY spectrum of **2** (500 MHz, DMSO-d_6):numbered double arrows indicate observed dipolar spatial correlations whose cross-peaks are marked with the same number on the structure.

Table 1

^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data for **1** and **2**.

	1 ($CDCl_3$)		**2** ($CDCl_3$)		**2** (DMSO-d_6)
Position	δ_C	δ_H	δ_C	δ_H	δ_H
1	143.57	-	154.75	-	
2	112.76	-	104.43	-	
3	111.34	-	109.79	-	
4	148.05	-	147.83	-	
5	123.14	8.15 (d, J=8.3)	123.50	8.27 (d, J=9,5)	8.21 (m)
6	126.41	7.45 (t, J=8.3)	129.79	7. 69 (m)	7.80 (m)
7	128.07	7.53 (t, J=8.3)	129.00	7.69 (m)	7.80 (m)
8	122.33	8.18 (d, J=8.3)	124.69	8.44 (d, J=9.5)	8.36 (m)
9	127.72	-	127.71	-	
10	125.82	-	128.61	-	
11	123.97	6.13 (d, J=9.6)	171.24	-	
12	125.25	5.56 (d, J=9.6)	-	-	
13	75.83	-	-	-	
14	25.49	1.59 (s)	-	-	
15	2873	1.62 (s)	-	-	
1´	183.53	-	182.53	-	
2´	135.77	-	133.37	-	
3´	136.60	-	137.41	-	
4´	182.26	-	184.83	-	
5´	126.74	8.10	126.82	8.11 (d, J=8.7)	8.01 (m)
6´	133.54	7.62	134.00	7.78 (m)	7.93 (m)
7´	133.54	7.68	134.11	7.78 (m)	7.93 (m)
8´	125.97	8.09	126.46	8.16 (d, J=7.5)	8.01 (m)
9´	133.23	-	132.60	-	
10´	132.06	-	133.52	-	
11´	67.91	6.40 (d, J=9.3)	68.26	6.51 (d, J= 9.3)	6.42 (d, J=9.0)
12´	117.66	5.43 (d, J=9.3)	117.98	5.60 (d, J= 9.3)	5.50 (d, J=9.0)
13´	141.95	-	142.80	-	
14´	26.01	1.55	26.40	1.68 (s)	1.63 (s)
15´	18.97	2.03	19.30	2.10 (s)	2.03 (s)
MeO-11	-	-	52.17	3.72 (s)	3.65 (s)
HO-1	-	-	-	11.28 (s)	10.88 (s)

Conclusion

Two prenylated naphthoquinone dimers were isolated from *L. microphylla*. Beside other spectroscopic techniques, extensive NMR studies allowed their identification as the known tecomaquinone I, previously isolated from *L. sidoides*, and a novel one we have designated microphyllaquinone.

The unambiguous assignment of all NMR data with all hydrogen and carbons was also performed.

The “alecrim-de-tabuleiro” should maybe have the same properties of *L. sidoides*, which shows a similar chemical composition.

Acknowledgments

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