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Assignments of ^{1}H And ^{13}C Resonance Signals in 2-Methoxy-4,5-Methylenedioxypropiophenone with the Assistance of 1D And 2D NMR Experiments*

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SUMMARY. In connection with our continuous investigation of *Piper* species, we have isolated 2-methoxy-4,5-methylenedioxypropiophenone from the roots of *Piper marginatum*. The literature reported the same compound isolated from leaves of the same species, but only ^{1}H NMR (60 MHz), UV, IV and EM were used in spectroscopic assignments. In the present paper we assign unambiguously the protons and carbons peaks with the assistance of 1D and 2D NMR experiments.

RESUMEN. "Asignación de señales de resonancia ^{1}H y ^{13}C de 2-metoxi-4,5-metilenpropiofenona con el auxilio de experimentos de RMN en una y dos dimensiones (1-D y 2-D)". En relación al trabajo de investigación desarrollado en especies pertenecientes al género *Piper*, hemos aislado 2-metoxy-4,5-metilendioxipropiofenona a partir de las raíces de *Piper marginatum*. La literatura cita el mismo compuesto en hojas de la misma especie, pero en la asignación estructural solamente fueron utilizados ^{1}H RMN (60 MHz), UV, IV y EM en las atribuciones estructurales. En el presente trabajo asignamos sin ambigüedades los valores de los desplazamientos químicos de hidrógenos y carbonos con la ayuda de experimentos de RMN en una y dos dimensiones (1D y 2D).

INTRODUCTION

Piper marginatum Jacq. *(Piperaceae)*, popularly known in the state of Paraíba (Brazil) as "malvaisco", is an important medicinal plant for the natives of the Amazon where the decoction of leaves is used against liver and vesicle diseases and as a tonic with carminative and antispasmodic action [1,2]. In Brazilian folk medicine the plant is reputed for its analgesic/antiinflammatory and hemostatic properties [3]. The extract of the leaves prepared in hot water is used to trat toothache, rheumatism, tumor and bleeding skin wounds [3,4]. The roots are used as diuretic [5]. In previous work on roots of Piper marginatum we isolated croweacin [6], apiole, isoasarone, pipermargine, marginatine [7] and N-isobutyl-2-trans-4-trans-octadienamide, the only amide detected in this plant [8]. In further experiments with the $CHCl_3$ extract of the roots we isolated 2-methoxy-4,5-methylenedioxypropiophenone previously reported from literature in *Piper marginatum* leaves and identified by NMR 60 MHz, IV, UV and EM, with no reference to biologic activity data [9]. Now we assigne unambignously the protons and carbons signals with the assistance of 1D and 2D NMR (^{1}H x ^{1}H and ^{1}H x ^{13}C) experiments.

MATERIAL & METHODS

Plant material

The roots of *Piper marginatum* Jacq. were collected on September 1993 near the city of João Pessoa, PB, Brazil. A voucher specimen (Agra 1500-JPB) was deposited at the Herbarium Lauro Pires Xavier of the Universidade da Paraíba.

KEY WORDS: Phenylpropanoids, *Piperaceae*, *Piper marginatum*, Propiophenones.
PALABRAS CLAVE: Fenilpropanoides, *Piperaceae*, *Piper marginatum*, Propiofenonas

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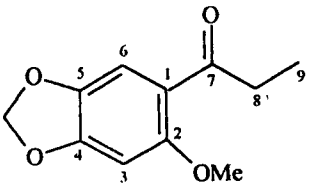
Extraction and Isolation

The powdered root (4.2 kg) was moistened with. NH_4OH (40% water solution) and extracted thrice with EtOH (10.0 L) at room temperature without agitation for three weeks. The resultant extract (170.0 g) was treated with 2% HCl and extracted three times with $CHCl_3$ (1.0 L). This extract (22.0 g) was then subjected to column cromatography (column 1, 80 x 7 cm) over silica gel, and consecutively eluted with hexane/$CHCl_3$/MeOH mixtures with increasing polarity and finally MeOH (100 fractions, 50 mL). The fractions 46-49 (10.0 g) corresponding to hexano/$CHCl_3$ 1:1 mixture were gathered and subjected to a second column (column 2, 100 x 4 cm) over silica gel using hexane-EtOAc (60 fractions, 25 mL). Fractions 8-11 subjected to preparative TLC developed with hexane-EtOAc (8:2) gave 2-methoxy-4,5-methylenedioxypropiophenone.



Equipment

All the NMR spectra were measured with a Bruker AC-300 spectrometer for $CDCl_3$ solutions, 300 MHz for ^1H and 75.46 MHz for ^{13}C NMR.

RESULTS AND DISCUSSION

The 300 MHz ^1H NMR spectrum showed the presence of two aromatic protons (δ 7.42, s, 1H and δ 6.55, s, 1H) and two aliphatic protons (δ 2.96, q, J = 7.2 Hz, 2H and δ 1.15, t, J = 7.2 Hz, 3H) in addition to two singlets for methoxy group (δ 3.87, s, 3H) and methylenedioxy group (δ 5.99, s, 2H). The shape of the signals for the two aromatic protons (singlet sharp) clearly pointing to a *para*-relationship between them. This seems to indicate that the methoxy group can be located on C-2. The $^1Hx^1H$ COSY spectrum showed cross peaks between the signals: δ 2.96/δ 1.15 and δ 3.87/δ 6.55. The two former cross peaks allows to assign : δ 2.96 and δ 1.15 to H-8 and H-9, respectively, and the two latter to methoxy group and H-3. The ^{13}C NMR spectrum (Proton-decoupled spectrum and DEPT 135°) showed the presence of 11 signals: two methylic (δ 9.01 and δ 56.61), two methylenic (δ 37.31 and δ 102.26) and two methynic (δ 94.28; δ 109.52), as well as five signals at δ 200.77; 156.67; 152.10; 141.60 and 120.26 not observed in the DEPT spectrum, attributed to non-protonated carbons. The highest frequency signal at δ 200.77 was attributed to the carbonyl carbon atom on the basis of chemical shift and heteronuclear correlation (^2J) with the resonance signal at δ 2.96 (H-8) in the HMBC spectrum.

A careful analysis of the non-protonated carbon signals in the aromatic region with the assistance of 2D heteronuclear experiment $^{2,3}J$ (C,H) couplings led us to stablish the resonance signal at δ 156.67 and δ 120.26 to the carbon-2 and carbon-1, on the basis of heteronuclear correlation (^3J) between the resonance signals δ 3.87/δ 156.67 and δ 2.96/δ 120.26, respectively. The two others resonance signals of the non-protonated carbons at δ: 152.10 and 141.60 were attributed to the carbons 4 and 5, respectively, on the basis of the following heteronuclear correlations: δ 6.55/δ 120.26, δ 141.60, δ 152.10, δ 7.42/δ 152.10, δ 156.67 and δ 5.99/δ 152.10, δ 141.60. Among these heteronuclear correlations the calculation of the chemical shifts for carbons in aromatic ring [10] allows to predict that C-4 is less shielded than C-5. The assignment to methynic (δ 109.52 and 94.28), methylenic (δ 37.31 and 102.26) and methylic (δ 9.01) carbons were achieved by means of a HETCOR experiment ^1J (C, H) couplings. The spectrum showed the following H-C correlations: δ 7.42/δ 109.52; δ 6.55/δ 94.28; δ 5.99/δ 102.26; δ 2.96/δ 37.31 and δ 1.15/δ 9.01 and allowed to assigne unambiguously these carbon peaks to 6, 3, 10, 8 and 9, respectively. Complete ^1H and ^{13}C NMR data for the compound are given in Table 1.

Since 2,4,5-trioxiphenylpropanoids as γ-asarone, α-asarone and asaricin has been reported to have antimicrobial activities against *Escherichia coli* and *Bacillus subtilis* [11], 2-methoxy-4,5-metylenedioxypropiophenone may be answerable for some activities showed by *Piper marginatum*.

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	H	^1J	^2J	^3J
1	—	120.26		
2	—	156.67		
3	6.55 (s, 1H)	94.28	152.10	120.26; 141.60
4	—	152.10		
5	—	141.60		
6	7.42 (s, 1H)	109.52	—	156.67; 152.10
7	—	200.77		
8	2.96 (q, J=7.2Hz, 2H)	37.31	200.77	
9	1.15 (t, J=7.2 Hz, 3H)	9.01		
OMe-2	3.87 (s, 3H)	56.26	—	156.67
CH_2O_2	5.99 (s, 2H)	102.26		

Table 1. ^1H and ^{13}C NMR ($CDCl_3$) data with correlations based on 2D heteronuclear experiment optimized for ^1J(C, H) and $^{2,3}J$(C, H) couplings.

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