Total assignment of the ^1H and ^{13}C NMR spectra of piperovatine

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Abstract. Total and unambiguous assignment of the ^1H NMR spectrum of piperovatine [6-(4-methoxyphenyl)-*N*-(2-methylpropyl)-2,4-hexadienamide] was carried out using conventional 1D NMR methods and spectral spin–spin simulation. Based on these data, the complete assignment of the ^{13}C NMR chemical shift values was made by a $^{13}\text{C}/^1\text{H}$ chemical shift correlation diagram and conventional considerations for the quaternary carbon atom.

1. Introduction

Piperovatine (**1**) [6-(4-methoxyphenyl)-*N*-(2-methylpropyl)-2,4-hexadienamide] is a naturally occurring alkaloid, which exhibits insecticidal [1] and local anesthetic activity [2,3]. It has been isolated mainly from several *Ottonia* [3–5] and *Piper* species [2,6]. Although syntheses of the alkaloid have been carried out [4,7–9], detailed NMR investigations of this interesting natural product have, to our knowledge, not been performed. The ^1H NMR spectrum of **1** has previously been reported [4,8] but the assignment of the spectral data remained ambiguous; besides, a ^{13}C NMR study has not been described. In this work we wish to report the unambiguous and complete assignment of the ^1H and ^{13}C NMR spectra of **1**, isolated during a phytochemical study of *Piper darrience*, which is used in the bolivian folk medicine against toothache.

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2. Experimental

2.1. Plant material

Piper darienense D.C. was collected in the neighborhood of the Blanco river, between Remancito and Cafetal, in the Beni department, Itenez province, Bolivia, in February 1996. It was classified by Dr. Stephan Beck from the National Herbarium of Bolivia, where a sample (voucher no. 4012) is deposited.

2.2. Extraction and isolation

Air dried roots (532 g) of *Piper darienense* D.C. were extracted with ethanol after removing the grease with petroleum ether. The ethanol was evaporated under vacuum and the residue was washed with CHCl_3 . The chloroform was evaporated and the residue chromatographed by column chromatography (CC) on silica gel 60 Å (Aldrich, 70–230 mesh). A fraction of 500 mL was collected using chloroform and then 8 fractions of 300 mL were collected with ethanol. The second ethanolic fraction was purified by TLC on silica gel (Fluka A.G., Buchs SG kieselgel GF₂₅₄) using chloroform–ethyl acetate (10 : 2 v/v). Compound **1** was obtained as a solid, which was crystallized from ethyl acetate (300 mg). It was further purified on column chromatography using silica gel 60 Å (Merck, 230–400 mesh) and chloroform–ethyl acetate (10 : 2 v/v). Crystallization from ethanol gave colorless needles, m.p. 121–122°. The identity of **1** was confirmed by comparing its physical and NMR spectral data with those reported in the literature [4].

2.3. Nuclear magnetic resonance instrumental conditions

The ^1H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, on a Varian XL-300GS spectrometer using CDCl_3 with TMS as the internal reference. Measurements were performed at ambient probe temperature using 5 mm o.d. sample tubes. For the $^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment, the standard pulse sequence was used [10,11]. The spectra were acquired with 1024 data points and 128 time increments with 128 transients per increment. The f_1 and f_2 spectral widths were 12048.2 and 2334.8 Hz, respectively. The relaxation delay was 1 s and an average $^1J(\text{C,H})$ was set to 140 Hz.

3. Results and discussion

In previous ^1H NMR spectral reports of **1** [4,8] H-2'', H-3, H-4 and H-5 are mentioned only as multiplets and the signals for the aromatic H-2', H-3', H-5' and H-6' are not assigned at all. Using conventional 1D NMR methods and spectral spin–spin simulation we describe herein the precise chemical shift and coupling constant values for each of these protons, as is shown in Table 1.

Inspection of the ^1H NMR spectrum of **1** showed at first glance the H-2'' signal as a septet. However, such a multiplicity is discarded because the intensity ratio between the higher intensity signals for a septet is $20/15 = 1.33$ in contrast with the spectrometer digitally readed signal intensities $135.485/107.925 = 1.255$, which clearly corresponds to a nonet ($70/56 = 1.25$). An amplitude increased plot shows that the H-2'' signal is found as a nonet ($J = 6.7$ Hz) centered at 1.79 ppm with intensity ratio 1 : 8 : 28 : 56 : 70 : 56 : 28 : 8 : 1, as expected for an isobutyl methine proton. A selective irradiation of this signal simplifies, from a double doublet to a doublet, the signal at 3.16 ppm assigned to the protons of the

Table 1
 ^1H and ^{13}C NMR spectral assignments of piperovatine (**1**)

Atom	^1H			^{13}C
	δ (ppm)	mult	J (Hz)	δ (ppm)
1				166.2
2	5.78	d	14.9	122.7
3	7.21	dd	14.9, 10.0	140.6
4	6.12	dd	15.2, 10.0	129.0
5	6.20	dt	15.2, 6.6	140.9
6	3.42	d	6.6	38.2
1'				131.1
2', 6'	7.08			129.5
3', 5'	6.84	AA'BB'		113.9
4'				158.1
1''	3.16	dd	6.7, 6.1	46.9
2''	1.79	nonet	6.7	28.6
3''	0.92	d	6.7	20.1
4''	0.92	d	6.7	20.1
MeO	3.79	s		55.2
NH	5.49	brs

Fig. 1. (a) Section of the ^1H NMR spectrum and (b) spin-spin simulation of piperovatine (**1**).

methylene group (H-1'') and from a doublet to a singlet, the signal at 0.92 ppm assigned to the protons of the methyl groups (H-3'' and H-4'').

The H-3 signal is shown at 7.21 ppm as a double doublet ($J = 14.9, 10.0$ Hz) through couplings with H-2 and H-4. These couplings were confirmed by selective irradiation.

Fig. 2. Heteronuclear $^{13}\text{C}/^1\text{H}$ chemical shift correlation diagram of piperovatine (1).

The frequencies for H-4 and H-5 appear superimposed in the range 6.08–6.25 ppm. The assignment of their individual resonance frequencies and multiplicities was achieved through selective homonuclear irradiations combined with spectral spin–spin simulation. In agreement with an inspection of **1**, and considering only vicinal couplings, the multiplicities for H-4 and H-5 must correspond to a double doublet and to a double triplet, respectively. Thus, irradiation of the signal at 7.21 ppm (H-3) simplified the double doublet signal at 6.12 ppm for H-4 to a doublet. On the other hand, irradiation of the signal at 3.42 ppm (H-6) simplified the double triplet signal at 6.20 ppm for H-5 to a doublet. In order to perform the precise assignment of the H-4 and H-5 signals, a spectral spin–spin simulation [12] was made. Therefore a six spins case for the H-2, H-3, H-4, H-5 and 2 H-6 nuclei was solved iteratively. The results fitted satisfactorily with the experimental data (RMS error frequency 0.13), as shown in Fig. 1.

The assignment of the aromatic H-2', H-3', H-5' and H-6' signals (AA'BB' pattern) was made on the basis of their chemical shift [13]. Thus the signal at 7.08 ppm was identified as belonging to H-2' and H-6', while the signal at 6.84 ppm was assigned to H-3' and H-5'.

The remaining proton resonance assignments for H-1'', H-3'', H-4'' and the NH group were confirmed by selective proton irradiations and the interchange with deuterium oxide.

The six proton doublet signal ($J = 6.7$ Hz) at 0.92 ppm assigned to H-3'' and H-4'' was simplified to a singlet by irradiation of the signal at 1.79 ppm (H-2''). The two proton double doublet signal ($J = 6.7, 6.1$ Hz) at 3.16 ppm assigned to H-1'' was simplified to a doublet by irradiation of the signal at 1.79 ppm (H-2'') and, on addition of deuterium oxide, it also collapsed to a doublet. The broad singlet at 5.49 ppm assigned to the NH group interchanged with deuterium oxide. The complete ^1H NMR data of **1** are given in Table 1.

Once the complete ^1H NMR spectrum of **1** had been assigned, a $^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment allowed the assignment of all protonated carbons (Fig. 2). The non-protonated C-1 carbonyl signal appears at 166.2 ppm and the aromatic carbons C-1' and C-4' were assigned at 131.1 and 158.1 ppm, respectively, by application of additivity relationships [13]. The ^{13}C NMR chemical shift values are summarized in Table 1.

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