^1H and ^{13}C NMR assignments of dihydropipataline, the main of four long-chain 1-(3,4-methylenedioxyphenyl)-alkanes from *Piper darienense* D.C.

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Dedicated to the memory of Dr. Piet Leclercq

Abstract. Four 1-(3,4-methylenedioxyphenyl)-alkanes having linear ten, eleven, twelve and fourteen carbon atom chains, found in the roots of *Piper darienense* D.C., were separated by HPLC and their structures determined by mass spectrometry and NMR spectroscopy. Conventional 1D NMR methods were used for ^1H chemical shifts assignment of the main compound dihydropipataline (**3**) [1-(3,4-methylenedioxyphenyl)-dodecane]. The ^{13}C NMR assignment was carried out using conventional considerations and 2D NMR techniques (HETCOR and FLOCK) in combination with spectral ^{13}C NMR simulation and *ab initio* DFT-GIAO NMR calculations.

1. Introduction

Plants belonging to the genus *Piper* have been studied widely due to their medicinal and economic importance. These phytochemical investigations led to the isolation of a number of physiologically active compounds [1]. In a previous paper we reported the isolation of piperovatine from the ethanolic extract of the roots of *Piper darienense* D.C. [2]. We now report the isolation, from the petroleum ether extract, and the structure determination of the four new natural products: dihydrojuvadecene (**1**) [1-(3,4-methylenedioxyphenyl)-decane], 1-(3,4-methylenedioxyphenyl)-undecane (**2**), dihydropipataline (**3**) [1-(3,4-methylenedioxyphenyl)-dodecane] and 1-(3,4-methylenedioxyphenyl)-tetradecane (**4**). Although

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compounds **1–3** have been obtained by catalytic hydrogenation of the unsaturated natural products with C_{10} (juvadece) [3], C_{11} [4] and C_{12} (pipataline) [5] alkenyl side chains, this is the first time that the saturated compounds are isolated from nature. Compound **1** has been prepared from juvadece, which is a natural product with biological activity as insecticide, acting as an insect juvenile hormone mimic [3]. In addition, compounds of the type **1–4** have been recognized as plant growth regulators [6].

1: $n = 8$ **3:** $n = 10$
2: $n = 9$ **4:** $n = 12$

In this work we also describe the ^1H and ^{13}C NMR assignments of the main compound **3** based on 1D and 2D NMR techniques together with spectral ^{13}C NMR simulation and *ab initio* DFT-GIAO (gauge including atomic orbitals [7]) NMR calculations at the BPW91/6-311G(d,p) and B3LYP/6-311++G(2d,p) levels on an *ab initio* DFT optimized molecular geometry. Although the ^{13}C NMR spectral study of **3** has been described [8], the signal assignment was based only on additivity relationships.

2. Experimental

2.1. General

Mass spectra (EIMS) were recorded at 20 eV on a Hewlett Packard 5989A spectrometer equipped with a Hewlett Packard 5890 Serie II Gas Chromatograph. The ultraviolet (UV) spectra were obtained on a Perkin-Elmer Lambda 12 spectrometer in EtOH. The high performance liquid chromatography (HPLC) separations were carried out on a Varian Associates Vista 5500 equipment. The column chromatographies (CC) were performed on activated neutral alumina (Merck, 70-230 mesh) and silica gel 60 Å (Aldrich, 70-230 mesh).

2.2. 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. A voucher specimen is in deposit at the National Herbarium of Bolivia (voucher no. 4012), where Dr. Stephan Beck identified the plant material.

2.3. Extraction and isolation

Air dried roots (532 g) of *Piper darrience* D.C. were extracted with petroleum ether. The solvent was evaporated under vacuum and the residue (5 g) was subjected to CC on silica gel (150 g). Elution with benzene provided six fractions (Fr) of 100 ml. Fr 1 and 2 were combined and percolated by CC on silica gel using 50 ml of petroleum ether–benzene (10:1, v/v). After removing the solvent, the residue was rechromatographed by CC on alumina (40 g). Elution with benzene afforded two fractions. The EIMS spectra and the gas chromatogram of the second fraction (0.8 g) showed a mixture of four structurally related compounds. The mixture was processed by reverse phase HPLC. The optimal chromatographic conditions were: 1 mg of sample in 10 μl of EtOH injected into a C_{18} reverse phase column (i.d. 4 mm, length 150 mm + 40 mm pre-column), using EtOH– H_2O (75:25, v/v) as the mobile phase at 1 ml/min and an UV detector operated at 287 nm. The peaks were collected after each of 30 successive runs. Each fraction was analyzed by EIMS and ^1H NMR spectroscopy, revealing the presence of **1** (5%, R_t = 14 min), **2** (2%, R_t = 27 min), **3** (89%, R_t = 36 min) and **4** (4%, R_t = 51 min).

1-(3,4-Methylenedioxyphenyl)-decane (1): EIMS m/z (rel. int.): 262 $[\text{M}]^{+\cdot}$ (5), 135 (100).

1-(3,4-Methylenedioxyphenyl)-undecane (2): EIMS m/z (rel. int.): 276 $[\text{M}]^{+\cdot}$ (7), 135 (100).

1-(3,4-Methylenedioxyphenyl)-dodecane (3): EIMS m/z (rel. int.): 290 $[\text{M}]^{+\cdot}$ (15), 135 (100); UV λ nm (log ϵ): 232 (3.7), 287 (3.6); ^1H and ^{13}C NMR see Table 1.

1-(3,4-Methylenedioxyphenyl)-tetradecane (4): EIMS m/z (rel. int.): 318 $[\text{M}]^{+\cdot}$ (7), 135 (100).

2.4. Nuclear magnetic resonance instrumental conditions

The ^1H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, from CDCl_3 solutions with TMS as the internal reference on a Varian Associates XL-300GS spectrometer. 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, a standard pulse sequence was used [9,10]. The spectra were acquired with 1024 data points and 128 time increments with 256 transients per increment. The f_1 and f_2 spectral widths were 10515.2 and 2344.7 Hz, respectively. The relaxation delay was 1 s and an average $^1J(\text{C,H})$ was set to 140 Hz.

The FLOCK experiment was performed using a described pulse sequence [11]. A collection of 256 time increments with 256 transients per increment in 1024 data points was made. The f_1 and f_2 spectral widths were 10952.9 and 2084.6 Hz, respectively. The relaxation delay D_1 was 1 s and Δ_1 , Δ_2 and Δ_3 were 0.05, 0.025 and 0.00357 s, respectively. The $^1J(\text{C,H})$ assumed in calculating the delay for the BIRD pulses was 140 Hz.

2.5. Calculations

A full geometry optimization for **3** was carried out with the *ab initio* DFT BPW91 and B3LYP methods using the 6-311G(d,p) and 6-31G(d,p) basis set, respectively. DFT-GIAO nuclear magnetic shielding calculations were performed at the BPW91/6-311G(d,p) and B3LYP/6-311++G(2d,p) levels. All calculations were carried out as implemented in the Gaussian98 program [12] on an SGI Origin 2000.

Table 1

¹H, ¹³C NMR spectral assignments and ¹³C/¹H correlations from a 2D-FLOCK experiment of dihydropipataline (**3**). Comparison of experimental (δC_{exp}), calculated (δC_{calc}) and predicted (δC_{pred}) ¹³C chemical shifts

Atom	Dihydropipataline (3)					Dodecane ^a
	¹ H δ (ppm), mult, <i>J</i> (Hz)	¹³ C δC_{exp} (ppm)	FLOCK correlations	¹³ C δC_{calc} (ppm) ^b	¹³ C δC_{pred} (ppm) ^c	¹³ C δ (ppm)
1	2.51, brt, 7.7	35.71	H-2'	34.72	34.35	13.99
2	1.55, m	31.76	H-1	33.14	29.22	22.67
3	1.25, brs	29.67 ^d	*	29.77	29.50	31.93
4	1.25, brs	29.21 ^d	*	29.49	29.08	29.36
5	1.25, brs	29.60 ^d	*	29.58	29.50	29.67
6	1.25, brs	29.65 ^d	*	29.64	29.65	29.71
7	1.25, brs	29.52 ^d	*	29.56	29.56	29.71
8	1.25, brs	29.67	*	29.71	29.67	29.67
9	1.25, brs	29.36	*	29.54	29.36	29.36
10	1.25, brs	31.93	H-12, *	30.93	31.93	31.93
11	1.25, brs	22.69	H-12	23.50	22.67	22.67
12	0.88, t, 6.7	14.11	not observed	13.70	13.99	13.99
1'		136.85	H-1, H-5'	136.23	134.55	
2'	6.67, d, 1.6	108.85	H-1, H-6'	106.52	109.16	
3'		147.44	H-2', H-5', H-7'	147.62	147.91	
4'		145.37	H-2', H-6', H-7'	146.02	145.49	
5'	6.72, d, 7.9	107.99	not observed	105.53	108.51	
6'	6.61, dd, 7.9, 1.6	121.00	H-1, H-2', H-5'	119.64	121.66	
7'	5.91, s	100.65	not observed	106.58	100.60	

*Correlation with the H-3 to H-11 signal at 1.25 ppm. ^aFrom [15]. ^bDerived from eqs. 3 and 3' (see Table 2). ^cACD Labs program [24]. ^dTentative assignment.

3. Results and discussion

Air dried roots of *Piper darrience* D.C. were extracted with petroleum ether. Column chromatographic separations of the extract, followed by reversal phase HPLC, afforded the four 1-(3,4-methylenedioxyphenyl)-alkanes **1–4**.

The EIMS spectrum of **3**, the main component, showed an $[M]^{+}$ peak at m/z 290 in agreement with the molecular formula C₁₉H₃₀O₂, and an intense fragment-ion peak at m/z 135 ascribed to the methylenedioxytropolium ion [13]. The ¹H NMR data of **3** (Table 1) evidenced the presence of the 3,4-methylenedioxyphenyl moiety by the three aromatic resonances characteristic for an aromatic 1,2,4-substitution pattern and the singlet for the methylenedioxy group H-7'. The spectrum also showed signals arising from benzylic and homobenzylic methylenes, nine aliphatic methylenes in a single signal and a terminal methyl group. Integration of these signals indicated that the substituent at position 1' was a twelve-carbon atom chain, a fact also supported by the mass spectrum. The proton resonance assignment for the homobenzylic methylene was confirmed after its selective proton irradiation at 1.55 ppm (H-2) which simplified the signal at 2.51 ppm, assigned to the benzylic methylene protons (H-1) from a triplet to a singlet. The ¹³C NMR data (Table 1) were also in agreement with structure **3**. They showed eighteen signals for the nineteen carbon atoms, six arising from aromatic carbons, eleven from the aliphatic carbons and one due to a methylenedioxy group. The assignment of the protonated carbons C-1, C-2, C-2', C-5', C-6', C-7' and C-12 was made from a ¹³C/¹H chemical shift correlation experiment. The non

protonated carbons C-1', C-3' and C-4' were assigned using substituent chemical shift (SCS) values [14]. Signal assignments of carbons C-8 to C-11 were made after comparison to those of dodecane [15] (Table 1). A 2D-FLOCK experiment [11] confirmed the assignments of the quaternary carbons C-1', C-3' and C-4' as well as those belonging to carbons C-1, C-2, C-10 and C-11 (Table 1).

To perform the complete ^{13}C NMR chemical shifts assignment of **3**, including the signals for aliphatic carbons C-3 to C-7, spectral ^{13}C NMR simulations and *ab initio* DFT-GIAO methods were applied. Recent applications of *ab initio* DFT-based methods have provided results accurate enough for the solution of NMR signal assignments [16–20]. In particular DFT-GIAO NMR calculations on molecules with saturated long-chains have not been reported. In the case of **3**, with a twelve-carbon atom side-chain, the calculation was carried out on the *ab initio* DFT optimized geometry using the widely utilized density functionals BPW91 [17–22] and B3LYP [21,23] at BPW91/6-311G(d,p)//BPW91/6-311G(d,p), B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d,p) and BPW91/6-311G(d,p)//B3LYP/6-31G(d,p) levels [(NMR calculation//geometry optimization)]. The calculated isotropic GIAO magnetic shieldings σ_{IMS} for the three DFT methods are summarized in Table 2. At first attempt theoretical calculated shifts (δC_{calc} , ppm) were obtained by subtraction of the calculated σ_{IMS} from that for the standard TMS $\delta C_{\text{calc}} = \sigma_{\text{IMS}(\text{3})} - \sigma_{\text{IMS}(\text{TMS})}$, however the rms error obtained for aliphatic side-chain carbons C-1, C-2 and C-8 to C-12 (~ 7 ppm) hardly seems adequate for a reliable assignment of the spectrum. Therefore the theoretical calculated chemical shifts (δC_{calc} , ppm) were obtained by linear regression analysis between the experimental ^{13}C chemical shifts (δC_{exp} , ppm) and σ_{IMS} , represented by the general equation $\delta C_{\text{exp}} = m\sigma_{\text{IMS}} + i$ [23]. The results were better described when two separate correlations were made, first for the side-chain and then for the methylenedioxy-phenyl group carbon atoms. The equations, correlation coefficients and statistical evaluation [averaged deviation (av. dev.) and rms error] are shown in Table 2.

According to the statistical evaluation, the best correlation for aliphatic side-chain carbons C-1, C-2, and C-8 to C-12 was obtained with isotropic shieldings calculated at the BPW91/6-311G(d,p)//B3LYP/6-31G(d,p) level. The δC_{calc} values derived from eqs. 3 and 3', for all carbons in **3**, are shown in Table 1. After direct comparison, fairly accurate results were observed between δC_{calc} and δC_{exp} of the unambiguous ^{13}C NMR assignments (Table 1). Therefore the δC_{calc} for C-3 to C-7 suggest that the experimental ^{13}C NMR resonances at 29.67, 29.21, 29.60, 29.65 and 29.52 belong to C-3, C-4, C-5, C-6 and C-7, respectively (Table 1).

The best correlation for carbons C-1' to C-7' of the methylenedioxyphenyl group was obtained using the higher B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d,p) level, mainly due to an important decrease in individual deviation of C-7' ($\Delta\delta = \delta C_{\text{calc}} - \delta C_{\text{exp}}$) (Table 2). In a paper dealing with the DFT-GIAO NMR study of fluorobenzenes [18], larger errors were exhibited by carbons with attached heteroatoms than by unsubstituted carbons, thus suggesting that going to higher basis sets might be required for more accurate predictions. The results obtained here for C-7' are in agreement with such an idea.

The predicted spectrum of **3** was obtained by a spectral database ^{13}C NMR simulation program [24] after introducing the experimental data of dodecane [15] and (8s,8's)-bis-(3,4-methylenedioxy)-8,8'-neolignan [25]. The predicted ^{13}C chemical shifts (δC_{pred} , ppm) are shown in Table 1. The δC_{pred} for carbons C-1, C-2, C-8 to C-12 and C-1' to C-7' are in good agreement with experimental values. In the case of carbons C-3 to C-7, only the δC_{pred} for C-4, C-6 and C-7 were consistent with those assigned by *ab initio* DFT-GIAO NMR calculation.

Compounds **1**, **2** and **4**, isolated only in traces, were identified by mass spectrometry; **1** showed an $[\text{M}]^{+}$ peak at m/z 262 consistent with the molecular formula $\text{C}_{17}\text{H}_{25}\text{O}_2$, **2** showed an $[\text{M}]^{+}$ peak at m/z 276 in agreement with $\text{C}_{18}\text{H}_{28}\text{O}_2$ and **4** showed an $[\text{M}]^{+}$ peak at m/z 318 consistent with

Table 2

Isotropic GIAO magnetic shieldings σ_{IMS} of **3** and statistical evaluation of linear correlation $\delta C_{\text{exp}} = m\sigma_{\text{IMS}} + i$

Atom	<i>ab initio</i> calculated σ_{IMS} (DFT-method//Geometry)					
	BPW91/6-311G(d,p)// BPW91/6-311G(d,p)		B3LYP/6-311++G(2d,p)// B3LYP/6-31G(d,p)		BPW91/6-311G(d,p)// B3LYP/6-31G(d,p)	
1	140.00		139.95		140.89	
2	141.72		140.47		142.90	
3	146.14		145.17		147.18	
4	146.43		145.54		147.53	
5	146.38		145.01		147.42	
6	146.25		145.38		147.34	
7	146.39		144.91		147.45	
8	146.18		145.32		147.26	
9	146.40		145.00		147.47	
10	144.65		143.90		145.71	
11	154.00		153.27		155.14	
12	166.50		166.13		167.59	
1'	43.36		36.89		44.47	
2'	73.04		70.40		74.58	
3'	31.57		26.36		32.93	
4'	33.26		28.43		34.55	
5'	74.07		70.98		75.58	
6'	60.00		57.31		61.28	
7'	72.87		75.17		74.51	
eqs.	1 ^a	1 ^b	2 ^a	2 ^b	3 ^a	3 ^b
<i>m</i>	−0.788 ^a	−0.993 ^b	−0.774 ^a	−0.902 ^b	−0.787 ^a	−0.987 ^b
<i>i</i>	145.0 ^a	179.1 ^b	142.2 ^a	171.1 ^b	145.6 ^a	180.1 ^b
<i>r</i>	−0.991 ^a	−0.988 ^b	−0.986 ^a	−0.997 ^b	−0.992 ^a	−0.989 ^b
av. dev.	0.73 ^a	2.02 ^b	0.96 ^a	1.08 ^b	0.69 ^a	1.93 ^b
rms	0.88 ^a	2.72 ^b	1.13 ^a	1.36 ^b	0.82 ^a	2.65 ^b
$\Delta\delta_{\text{max}}$	6.07 (C-7')		2.68 (C-7')		5.93 (C-7')	

^aData correlation of side-chain carbons C-1, C-2 and C-8 to C-12.^bData correlation of methylenedioxyphenyl group carbons C-1' to C-7'.

$\text{C}_{21}\text{H}_{34}\text{O}_2$. In addition, the EIMS spectra of the three compounds showed an intense peak at m/z 135, ascribed to the methylenedioxytropolium ion [13]. These data confirmed both the presence of a 3,4-methylenedioxyphenyl moiety in **1**, **2** and **4** and the presence of a ten, an eleven and a fourteen-carbon side chain, respectively. The ^1H NMR spectra of **1**, **2** and **4** appeared virtually identical to that of **3**, the difference being in the integral of the signal at 1.25 ppm.

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