

Hydrolysis of Methyl Benzoate from *Piper arboreum* by *Naupactus bipes* Beetle

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O 3-geranil-4-hidroxibenzoato de metila (**1**), um novo produto natural, foi isolado das folhas de *Piper arboreum* (Piperaceae). O metabolismo das folhas de *P. arboreum* pelo besouro *Naupactus bipes* (Germar, 1824 - Coleoptera: Curculionidae) resultou na biotransformação de **1** para o ácido 3-geranil-4-hidroxibenzoico (**2**). As estruturas dos metabólitos **1** e **2** foram determinadas com base na interpretação dos dados espectroscópicos de EM, IR, RMN de ^1H e de ^{13}C .

A new natural product was isolated from *Piper arboreum* (Piperaceae) leaves, the methyl 3-geranyl-4-hydroxybenzoate (**1**). The metabolism of *P. arboreum* leaves by *Naupactus bipes* beetle (Germar, 1824 - Coleoptera: Curculionidae) led to the hydrolysis of **1** to 3-geranyl-4-hydroxybenzoic acid (**2**). The structures of both compounds were determined based on spectroscopic analysis (^1H and ^{13}C NMR, MS, and IR).

Keywords: *Piper arboreum*, prenylated benzoic acid, piperaceae, biotransformation, *Naupactus bipes*

Introduction

Piper arboreum is a shrub with approximately 3 m height and it is popularly known as long pepper, rosemary-of-Angola or wood-of-Angola.¹ *P. arboreum* has been used in Brazil as tea and for treatment of rheumatism, bronchitis, colds and flu.² A previous chemical investigation carried out on leaves of *P. arboreum* led to the identification of the amides *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*),9(*Z*)-pentadienoyl]-pyrrolidine, arboreumine, *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*)-pentaenoyl]-pyrrolidine and *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*),9(*E*)-pentadienoyl]-pyrrolidine.³ Additional investigation on leaves from allopatric species of *P. arboreum* revealed now the occurrence of a new natural product methyl 3-geranyl-4-hydroxybenzoate (**1**) previously described as synthetic product.^{4,5} The function of plant secondary metabolites in mediating insect-plant interactions has been addressed in several studies^{6,7} and more recently, the specificity of *Piper*-insects relationship and the metabolism of secondary compounds by herbivorous insects has been investigated.^{8,9} Herein, we describe the hydrolysis of methyl benzoic acid derivative **1** to 3-geranyl-4-hydroxybenzoic acid (**2**) during the digestive process of *P. arboreum* leaves by *N. bipes* beetles.

Experimental

Instrumentation and chromatography materials

IR spectra were measured in KBr pellets on a Perkin-Elmer infrared spectrometer model 1750. UV spectra were recorded on a HP 8452 A spectrophotometer using MeOH as solvent. LREIMS spectra were measured at 70 eV and recorded on a VG Platform II spectrometer. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on Bruker DPX-300 spectrometer. CDCl_3 (Aldrich) was used as solvent and TMS as internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (*J*) in Hz. Elemental analyses were obtained on a Perkin-Elmer elemental analyzer model 2400 CHN. Silica gel (Merck, 230-400 mesh) was used for column chromatographic separations, while silica gel 60 PF254 (Merck) was used for analytical (0.25 mm) or preparative (1.0 mm) TLC chromatography. HPLC analysis of extracts and pure compounds were performed on a HP-1050 instrument using a reversed phase column Supelco C18 eluted in a gradient mode starting with MeOH:H₂O (1:1) raising to 100% MeOH in 30 min, with a flow rate of 1 mL min⁻¹ and detection set at 254 nm.

Plant material

Leaves from *P. arboreum* were collected on the Campus of the Universidade de São Paulo, Brazil (S 23°33'915");

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W 46°43'671"), and identified by Dr. Elsie F. Guimarães (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Brazil). The voucher specimen (Kato-0697) was deposited in the Herbarium of Instituto de Botânica, São Paulo, Brazil.

Insect material

Specimens of *N. bipes* that had been feeding on *Piper* species were collected at the Campus of the Universidade de São Paulo in December 2000 and identified by Dr. Sérgio Antônio Vanin (Departamento de Zoologia, Instituto Biociências da Universidade de São Paulo, São Paulo, Brazil). Voucher specimen (CSR 001) has been deposited at Museu de Zoologia da Universidade de São Paulo. Adult beetles were reared in cages in the laboratory and maintained on a diet constituted of *P. arboreum* leaves under artificial light (17 h light-7 h dark) at ambient temperature (24-27 °C).

Extraction and isolation of constituents

Dried ground leaves (40 g) from *P. arboreum* were extracted three times with CH₂Cl₂-MeOH (2:1) (2 × 500 mL), which after concentrated in vacuum yielded 12 g of crude extracts. Part of the extract from leaves (5 g) was suspended in MeOH-H₂O (1:4), filtered in a bed of celite (2/3, v/v), then 5 mL of 1 mol L⁻¹ NaCl solution was added to the filtrate and the solution was extracted three times with CH₂Cl₂ (150 mL each). The organic fraction was dried over anhydrous Na₂SO₄ and concentrated in vacuum yielding 1 g of a chlorophyll free leaves extract. This extract was subjected to fractionation over silica gel column using hexane with increasing amounts of EtOAc as eluent yielding 30 fractions (1-30). Fraction 5 (50 mg) was subjected to prepare TLC hexane:EtOAc:AcOH (4:1:0.5), three elutions, to yield **1** (16 mg).

The dried feces (400 mg) of *N. bipes* feeding on *P. arboreum* leaves were extracted three times with CH₂Cl₂-MeOH (2:1) (60 mL each). The residue was concentrated in vacuum yielding 65 mg of crude extract. The extract was subjected to prepare TLC using hexane:EtOAc:AcOH (3:6.7:0.3), three elutions, to yield **2** (1.3 mg).

Methyl 3-geranyl-4-hydroxybenzoate (1)

White amorphous solid; IR (KBr) ν_{\max} /cm⁻¹: 3368, 2957, 2920, 1713, 1603, 1507, 1434, 1202, 1198 and 772; LREIMS *m/z* (rel. int.) 288 [M] (2), 175 (48), 123 (67), 69 (78) and 41 (100). Elemental analysis: C 74.59%, H 8.45%, O 16.96%; calculated for C₁₈H₂₄O₃: C 74.97%, H 8.39%, O 16.64%; ¹H and ¹³C NMR, see Table 1.

Table 1. ¹H and ¹³C NMR (300 and 75 MHz, respectively, CDCl₃, *J* in Hz) data for compound **1**

Position	δ ¹ H	δ ¹³ C
1		122.6
2	7.80 (1H, d, 2.2)	131.9
3		126.7
4		158.8
5	6.83 (1H, d, 8.9)	115.7
6	7.83 (1H, dd, 8.9, 2.2)	129.7
1'	3.39 (2H, d, 7.15)	39.7
2'	5.31 (1H, td, 7.15, 1.18)	123.7
3'		139.3
4'	2.12 (2H, m)	26.4
5'	2.12 (2H, m)	29.7
6'	5.07 (1H, m)	121.0
7'		132.1
8'	1.67 (3H, s)	25.7
9'	1.77 (3H, brs)	17.7
10'	1.59 (3H, s)	16.3
CO ₂ CH ₃	3.87 (3H, s)	51.8
CO ₂ CH ₃		167.1
OH	5.80 (1H, s)	

3-Geranyl-4-hydroxybenzoic acid (2)

White amorphous solid; LREIMS *m/z* (rel. int.) 274 [M] (1), 175 (16), 123 (48), 69 (85) and 41 (100). ¹H and ¹³C NMR, IR in agreement with literature data.^{10,11}

Results and Discussion

Compound **1** was isolated from extract (CH₂Cl₂-MeOH, 2:1) of *P. arboreum* leaves as a white amorphous solid. Its molecular formula was established as C₁₈H₂₄O₃ by elemental analysis and LREIMS data. The IR spectrum indicated the presence of hydroxyl group (3368 cm⁻¹), a carbonyl group (1713 cm⁻¹), an aromatic ring (1507, 1434 cm⁻¹), and C-O stretching band (1202 cm⁻¹). The ¹H NMR spectrum (Table 1) of **1** showed signals corresponding to three-coupled aromatic resonance at δ 7.80 (1H, d, *J* 2.2 Hz), 6.83 (1H, d, *J* 8.9 Hz) and 7.90 (1H, dd, *J* 8.9 and 2.2 Hz) assignable for a 1,4,5-trisubstituted aromatic ring and a signal at δ 5.80 (1H, s) characteristic of a phenolic hydrogen; it also displayed a singlet at δ 3.87 (3H, s) of a methyl ester group, which was confirmed by signal at δ_c 51.8. The assignments of ¹H and ¹³C NMR data signals for aromatic moiety were further confirmed by comparison to those reported for known benzoic acids analogues. The isoprenyl side chain signals were determined from COSY and HETCOR spectra. Thus, the methylene protons H-1' at δ 3.39 (2H, d, *J* 7.2 Hz) were coupled to the olefinic proton H-2' at δ 5.31 (1H, td, *J* 7.2 and 1.8 Hz), which in turn showed an allylic coupling to methyl group H-9' at δ 1.77 (3H, s). The methine proton H-6' at δ 5.07 (1H, m) was further coupled to the methylene proton H-5' at δ 2.12

(2H, m). The *E* geometry of the $\Delta^{2,3'}$ double bond was established based on ^{13}C NMR data of the vinyl methyl group C-9' (δ 17.7) when compared to the chemical shifts of geraniol (C-Me, δ 17.1) and nerol (C-Me, δ 24.0), which have (*E*) and (*Z*) configurations, respectively.¹² The assignments of aromatic carbons signals were based on comparison of ^1H and ^{13}C NMR data to those reported for known benzoic acid analogues.¹³ The linkage position of monoterpene moiety was deduced from NOESY spectrum and also by comparison of ^1H and ^{13}C NMR spectral data with those published for prenylated benzoic acids.^{14,15}

N. bipes beetle was observed feeding on *P. arboreum* leaves specimens growing in a cultivation area. Thus, ten individuals of beetle were collected and maintained during three weeks on a diet constituted of fresh leaves

of *P. arboreum* in order to obtain fecal material from *N. bipes*. The collected feces were dried and extracted with $\text{CH}_2\text{Cl}_2:\text{MeOH}$ and the same procedure was carried out with not predated leaves from host plants. The fecal and leaves extracts were analyzed by HPLC and the chromatographic profile of fecal extract showed an additional major compound as compared to the chromatogram of leaves extracts. Moreover, a decrease in the relative content of **1** in the fecal extract chromatogram as compared to leaves extract chromatogram was observed and a new compound **2** could be observed (Figure 1). The fecal extract was subjected to purification steps resulting in isolation of **2**. Its structure was established based on spectroscopic data of LREIMS, ^1H and ^{13}C NMR as the known 3-geranyl-4-hydroxybenzoic acid previously isolated from *P. gaudichaudianum* and *P. elongatum*.^{10,11}

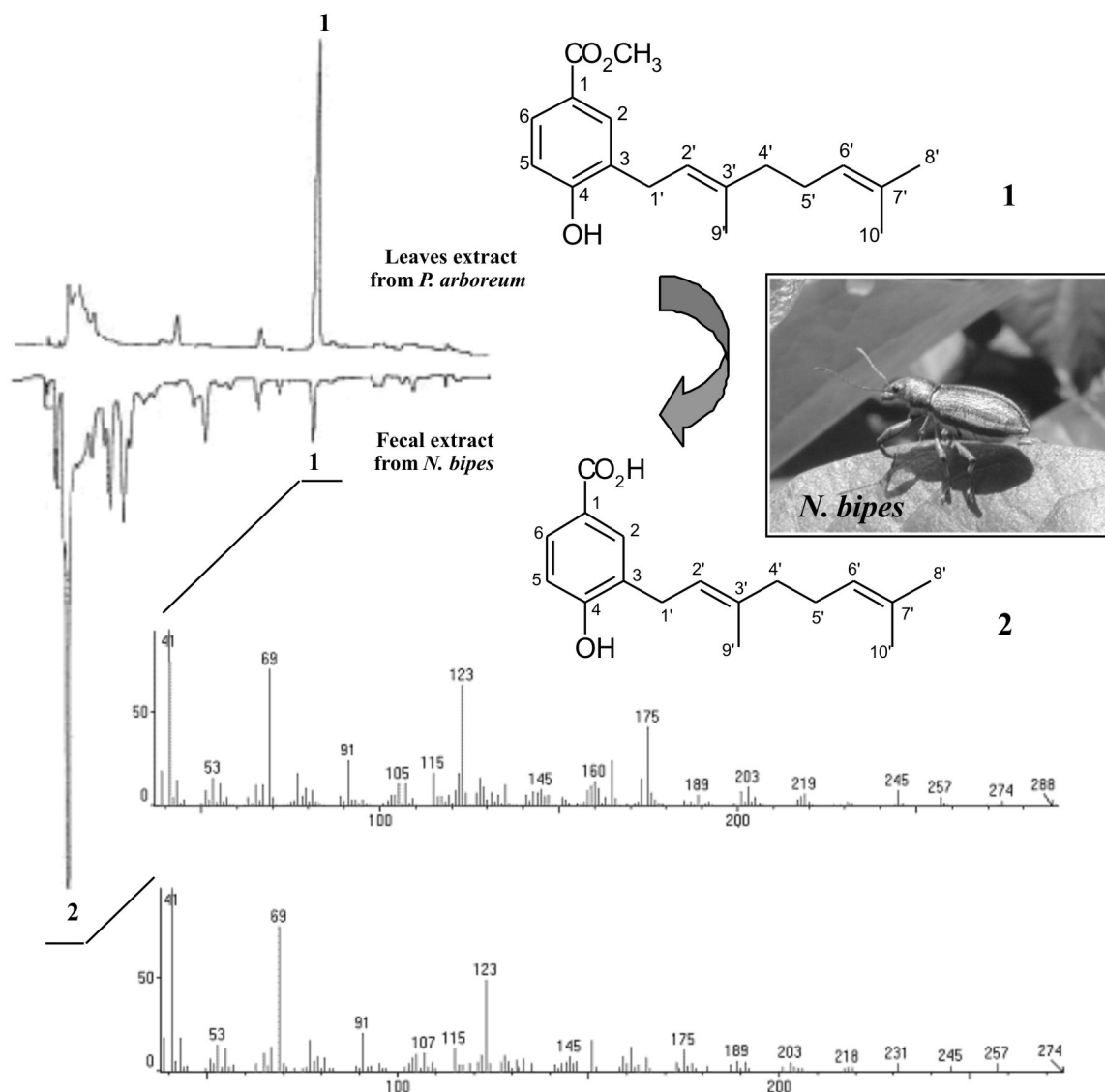


Figure 1. Chromatogram (HPLC) of extract from leaves of *P. arboreum* and fecal extract from *N. bipes* beetle. Mass spectra (LREIMS) of **1** and **2** ($\text{C}_{18}\text{H}_{24}\text{O}_3$, $M = 288$ Da; $\text{C}_{17}\text{H}_{22}\text{O}_3$, $M = 274$ Da, respectively).

This finding is compatible with the formation of benzoic acid **2** as hydrolysis product of **1** during digestive process of beetle *N. bipes*. A previous investigation carried out on *N. Bipes* feeding on *Piper solmsianum* leaves revealed the *O*-demethylation reaction at both *para* positions of 3,4,5-trimethoxyphenyl ring of tetrahydrofuran lignan (-)-grandisin and no trace of benzoic acid **2** could be detected in their feces.⁹

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Supplementary Information

Supplementary data are available free of charge at <http://jbc.sbq.org.br>, as PDF file.

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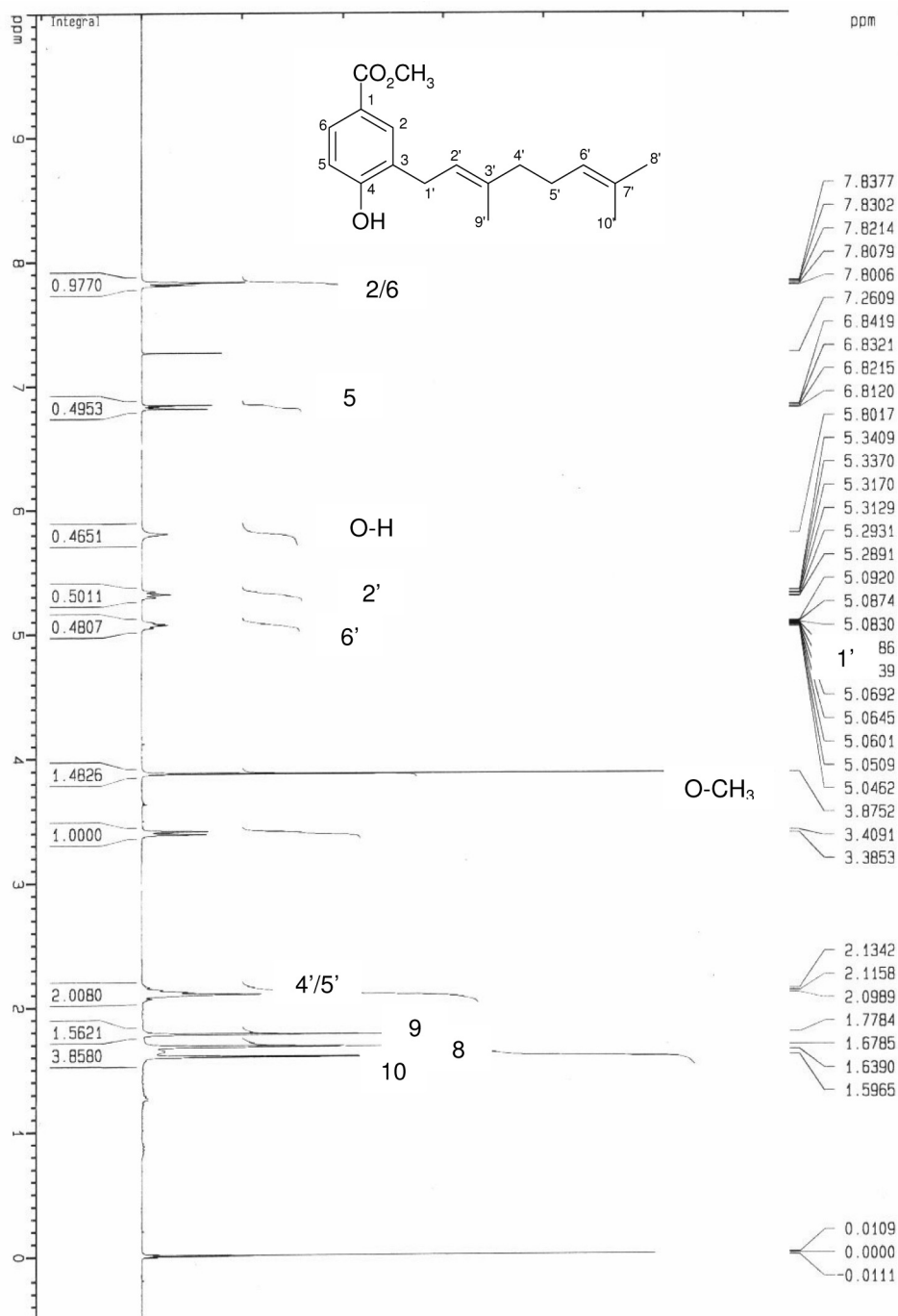


Figure S1. ¹H NMR spectra of compound **1** (300 MHz, CDCl₃, TMS).

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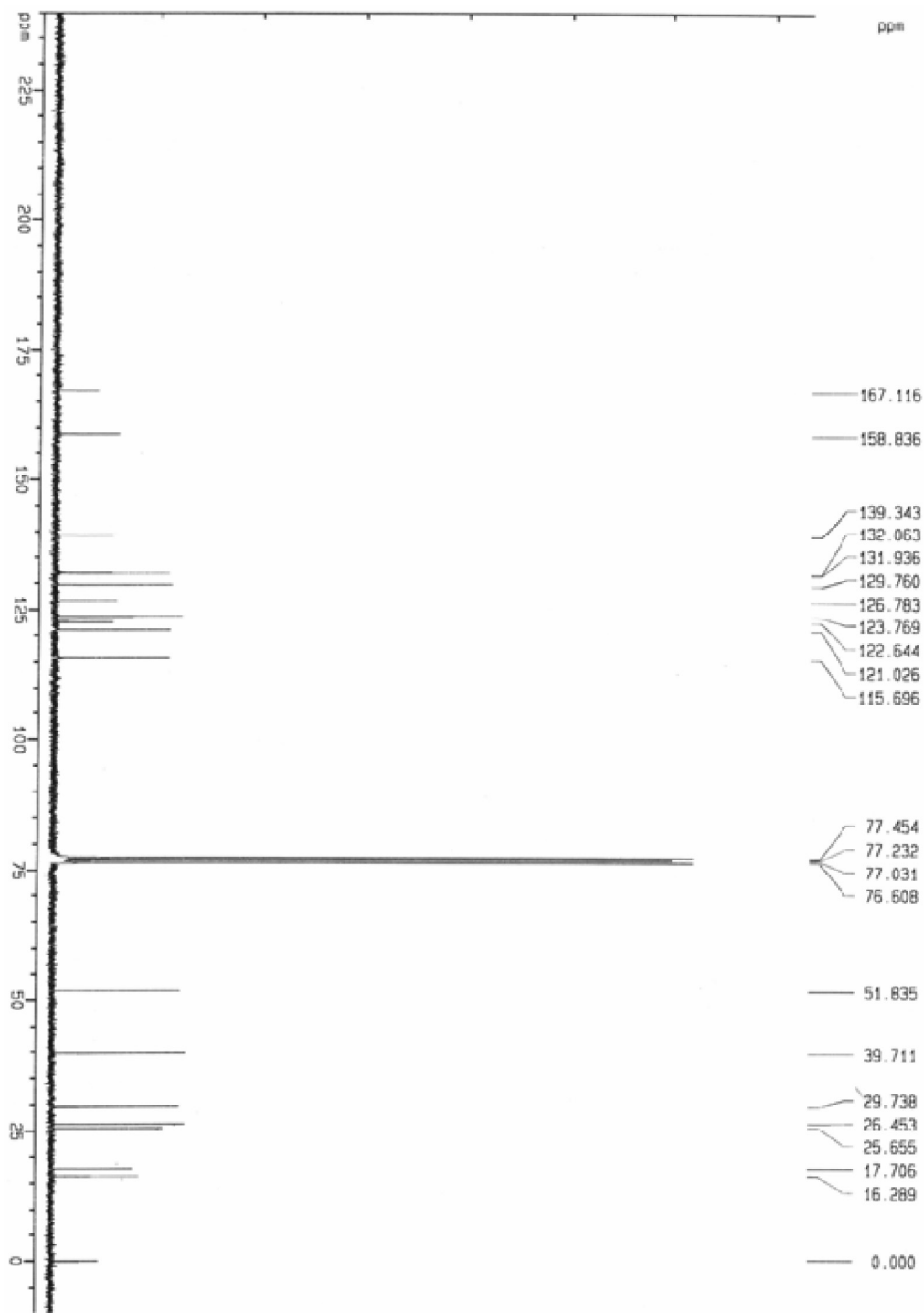


Figure S2. ¹³C NMR spectra of compound 1 (75 MHz, CDCl₃, TMS).

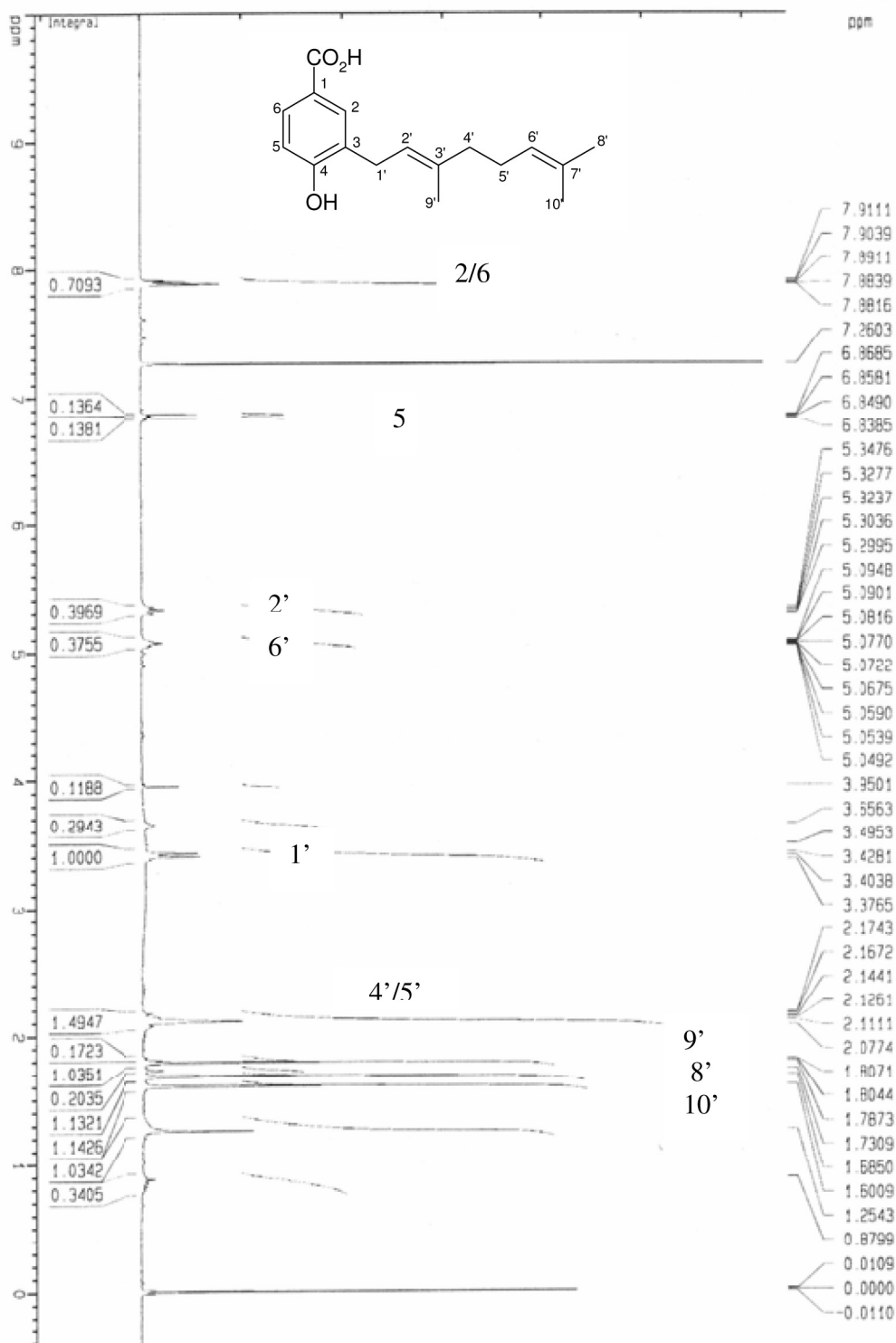


Figure S3. ¹H NMR spectra of compound 2 (300 MHz, CDCl₃, TMS).

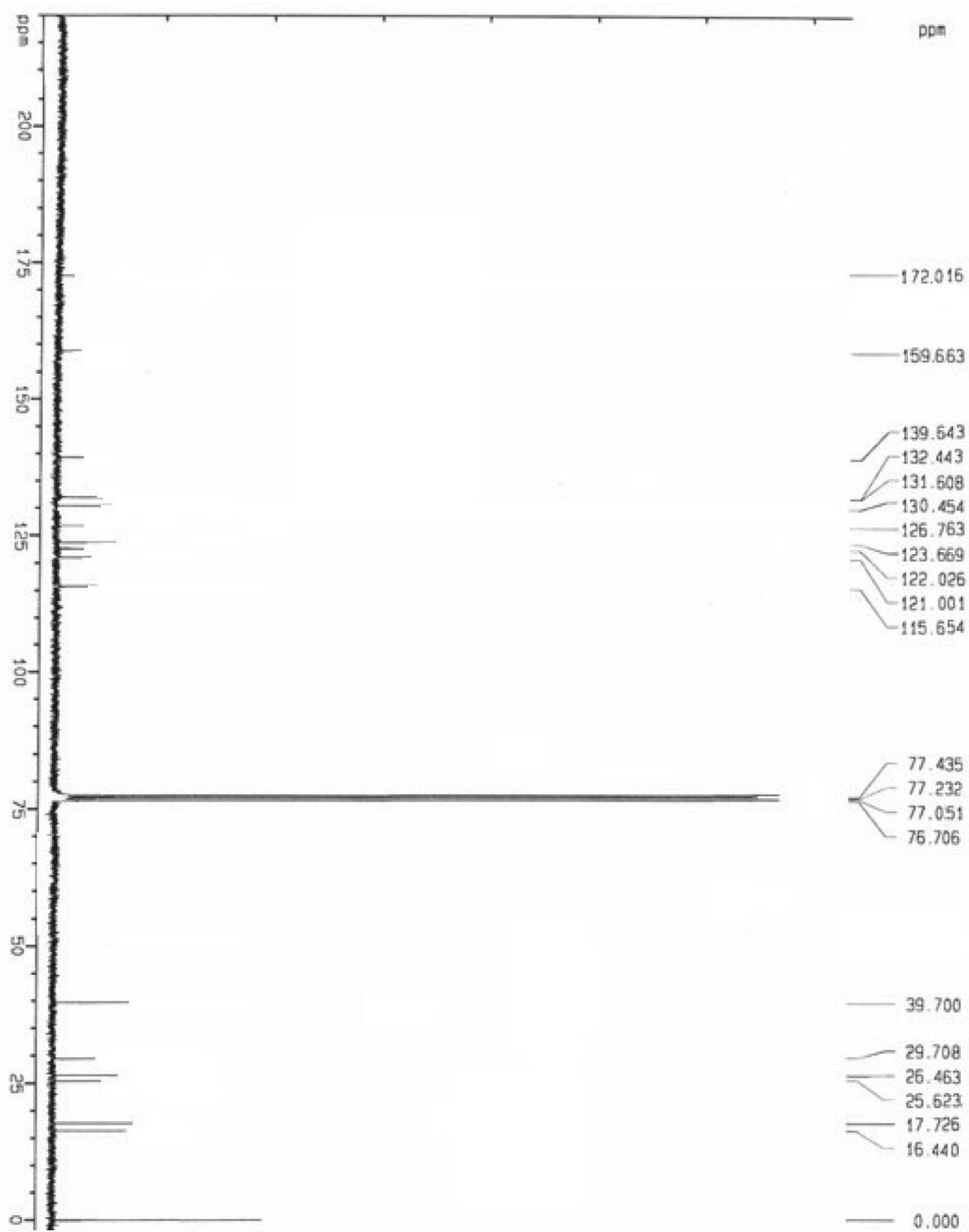


Figure S4. ¹³C NMR spectra of compound 2 (75 MHz, CDCl₃, TMS).