

Abietane Diterpenes from *Hyptis crassifolia* Mart. ex Benth. (Lamiaceae)

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O estudo fitoquímico do extrato etanólico das raízes de *Hyptis crassifolia* Mart. ex Benth. (Lamiaceae) levou ao isolamento e elucidação estrutural de nove diterpenos identificados como 11,12,15-tri-hidroxi-8,11,13-abietatrien-7-ona, 6 α ,11,12,15-tetra-hidroxi-8,11,13-abietatrien-7-ona, 11,12,16-tri-hidroxi-17(15→16)-abeo-abieta-8,11,13-trien-7-ona, (16S)-12,16-epoxi-11,14-di-hidroxi-17(15→16)-abeo-abieta-8,11,13-trien-7-ona, incanona, ferruginol, sugiol, óxido de 11-oxomanoíla e óxido de 11 β -hidroximanoíla. Os compostos 11,12,16-tri-hidroxi-17(15→16)-abeo-abieta-8,11,13-trien-7-ona e 6 α ,11,12,15-tetra-hidroxi-8,11,13-abietatrien-7-ona são inéditos na literatura, a 11,12,15-tri-hidroxi-8,11,13-abietatrien-7-ona está sendo relatada pela primeira vez como um novo diterpeno abietano natural, enquanto que para a (16S)-12,16-epoxi-11,14-di-hidroxi-17(15→16)-abeo-abieta-8,11,13-trien-7-ona, isolada anteriormente de *Teucrium divaricatum* Subsp. *villosum*, propõe-se uma revisão dos dados de ressonância magnética nuclear (NMR) ¹H e ¹³C. A determinação estrutural de todos os constituintes foi realizada através de técnicas espectroscópicas como espectrometria de massas de alta resolução (HRMS), infravermelho (IR), NMR de ¹H e ¹³C, incluindo sequências de pulsos uni e bidimensionais, e comparação com dados descritos na literatura.

The phytochemical study of the ethanol extract from roots of *Hyptis crassifolia* Mart. ex Benth. (Lamiaceae) led to the isolation and structure elucidation of nine diterpenes identified as 11,12,15-trihydroxy-8,11,13-abietatrien-7-one, 6 α ,11,12,15-tetrahydroxy-8,11,13-abietatrien-7-one, 11,12,16-trihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one, (16S)-12,16-epoxy-11,14-dihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one, incanone, ferruginol, sugiol, 11-oxomanoyl oxide and 11 β -hydroxymanoyl oxide. Compounds 11,12,16-trihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one and 6 α ,11,12,15-tetrahydroxy-8,11,13-abietatrien-7-one are new, 11,12,15-trihydroxy-8,11,13-abietatrien-7-one is being reported for the first time as a new natural abietane diterpene, while for (16S)-12,16-epoxy-11,14-dihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one, previously isolated from *Teucrium divaricatum* Subsp. *villosum*, a revision of the ¹H and ¹³C nuclear magnetic resonance (NMR) data previously reported is proposed. Structure determination of all constituents was performed by mean of spectroscopic techniques such as high resolution mass spectrometry (HRMS), infrared (IR), ¹H and ¹³C 1D and 2D NMR and comparison with literature data.

Keywords: *Hyptis crassifolia*, Lamiaceae, abietane diterpenes, rearranged abietane diterpenes, labdane diterpenes

Introduction

Lamiaceae consists of about 258 genera and 6970 species distributed around the world,¹ mainly centered in the Mediterranean region despite reports of their occurrence in Australia and South America.² The *Hyptis* genus includes

around 400 species distributed throughout the Americas, West Africa, Fiji Island (Oceania), and western India.³ They are found in Northern and Northeastern Brazil, especially in the cerrado.⁴ Plants from this genus are used in folk medicine, especially in the treatment of fever, colds, asthma, headache, cramps, gastrointestinal infections, rheumatism, skin diseases and malaria, and also have antibacterial, antifungal and insect repellent properties.²

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Previous studies on the chemical constitution of *Hyptis* species report the isolation of triterpenoids,⁵ flavonoids,^{6,7} lignans,^{7,8} α -pyrone derivatives^{9,10} and diterpenoids,¹¹⁻¹⁴ namely labdanes and abietanes. The abietane diterpenes have attracted particular attention, due to their biological activities, in particular as antioxidant,¹⁵⁻¹⁷ antibacterial,¹⁸⁻²⁰ cytotoxic,²¹⁻²⁴ antiviral^{25,26} and antimalarial.²⁷ Among the diterpenes that exhibit these activities, were observed several highly oxygenated compounds that can present either the C-ring saturated or unsaturated, aromatic, and also with an *ortho* or *para*-naphthoquinone pattern. Previous works carried out on plants of the genus *Hyptis* from the Northeast of Brazil flora, reported the isolation of many abietane diterpenes, for example those isolated from roots of *H. martiusii*,¹¹ *H. platanifolia*¹² and *H. carvalhoi*.²⁸

In this paper, it is reported the phytochemical analysis of the ethanol extract from roots of *Hyptis crassifolia* Mart. ex Benth., a small shrub that grows in abundance in the state of Bahia to which no phytochemical investigation has been reported so far. This study led to the isolation and structure elucidation of nine diterpenes: four abietanes (**1**, **2**, **6** and **7**), three rearranged abietanes (**3-5**) and two labdanes (**8** and **9**). Compounds **2** and **3** have not yet being reported in the literature, whereas **1** is being reported for the first time from a natural source. A revision of the previously published nuclear magnetic resonance (NMR) data in the literature²⁹ is being proposed for compound **4**.

Experimental

General procedures

Melting points were measured on a digital Marconi MA-381 apparatus and are uncorrected. Optical rotations $[\alpha]_D^{20}$ were determined with a Jasco P-2000 digital polarimeter, operating with tungsten lamp at a wavelength of 589 nm at 20 °C. Infrared (IR) spectra were recorded using a Perkin-Elmer FTIR 100 spectrometer using the universal attenuated total reflectance accessory (UATR). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were performed with a SHIMADZU LCMS-IT-TOF (225-07100-34) equipped with a Z-spray ESI (electrospray) source operating either in the negative or positive mode. ¹H and ¹³C NMR (1D and 2D) spectra were performed on Bruker Avance DPX-300 and/or DRX-500 spectrometers equipped with 5 mm direct probe or inverse detection Z-gradient probe, respectively. ¹H NMR (300.13 and 500.13 MHz) and ¹³C NMR (75.47 and 125.75 MHz) spectra were measured at 25 °C. Chemical shifts (δ), expressed in parts *per million* (ppm), are referenced by the signal of the residual non-deuterated hydrogens

(¹H NMR) and the central peak of carbon-13 (¹³C NMR) of the deuterated solvents. Flash column chromatography and column chromatography (CC) were carried out on silica gel 60 A (Whatman, 70-230 mesh) and silica gel 60 A (Acros Organic, 0.035-0.070 mm), respectively. Thin layer chromatography (TLC) was performed on precoated silica gel polyester sheets (Kieselgel 60 F₂₅₄, 0.20 mm, Merck) by detection with a spray reagent of vanillin/perchloric acid/EtOH solution followed by heating at 100 °C. Normal phase semi-preparative HPLC separations were performed with a Shimadzu LC-20AT pump, UV-PDA (SPD-M20A) detector and WATERS-1525 pump, UV-PDA (WATERS 2996) detector, a Phenomenex Silica column (10 × 150 mm, 5 μ m particle size), with a flow rate of 4.72 mL min⁻¹ and column oven temperature 40 °C, monitoring at 273.8 nm.

Plant material

The roots of *Hyptis crassifolia* Mart. ex Benth. were collected in July 2010, at Mucujê county (Diamantina Plateau, Bahia State). The plant material was identified by Prof Maria Lenise Silva Guedes of Instituto de Biologia, Departamento de Botânica, Universidade Federal da Bahia. A voucher specimen (No. 95.183) is deposited in the Herbário Alexandre Leal Costa of the Departamento de Botânica, Universidade Federal da Bahia.

Extraction and isolation

The roots (875 g) of *Hyptis crassifolia* Mart. ex Benth., dried and crushed were macerated with EtOH (3 × 6.0 L), after extraction with hexane, at room temperature after standing for 72 h. The ethanol solution was concentrated under reduced pressure at 50 °C to yield the respective extract (26.0 g). The EtOH extract (22.0 g) was coarsely fractionated over a silica gel column using hexane/CH₂Cl₂ (1:1), CH₂Cl₂, CH₂Cl₂/EtOAc (1:1), EtOAc and MeOH as eluents. The hexane/CH₂Cl₂ (1:1) fraction (674.4 mg) was chromatographed on a silica gel column with hexane, hexane/CH₂Cl₂ (9:1, 7:3, 1:1), CH₂Cl₂ and MeOH to give 36 fractions (10 mL each), that after TLC analysis were pooled to 9 fractions (F_{1HD}-F_{9HD}). F_{4HD} (52.8 mg, 7:3 hexane/CH₂Cl₂) was separated through HPLC using a 9:1 hexane/EtOAc as eluent (v/v), with an injection volume of 200 μ L, under isocratic conditions to yield compound **6** (6.0 mg), $[\alpha]_D^{20} +25.7$ (c 0.2, CHCl₃) {lit. $[\alpha]_D^{20} +42.9^\circ$ (c 0.2, CHCl₃)}.³⁰ F_{6HD} (60.2 mg, hexane/CH₂Cl₂ 7:3) after successive recrystallizations in MeOH yielded 18.5 mg of **8** in the form of colorless crystals, mp 70.7-73.4 °C, $[\alpha]_D^{20} -33.8^\circ$ (c 0.2, CHCl₃) {lit. mp 96-97 °C, $[\alpha]_D^{20} -103.2^\circ$ (c 0.2, *i*-PrOH)}.³¹ Compound **4** (6.2 mg), a yellow solid,

was obtained from F_{8HD} (85.0 mg, 1:1 hexane/CH₂Cl₂), after semi-preparative HPLC analysis (mobile phase 98:2 hexane/isopropanol). The CH₂Cl₂ fraction (1.26 g) was chromatographed over silica gel by elution hexane/CH₂Cl₂ (1:1, 3:7), CH₂Cl₂, CH₂Cl₂/EtOAc (1:1), EtOAc and MeOH to give 32 fractions (10 mL each), that were combined into nine resulting fractions after TLC analysis (F_{1D}-F_{9D}). F_{4D} (110.5 mg, 1:1 hexane/CH₂Cl₂) was submitted to semi-preparative HPLC analysis, using hexane/EtOAc, 8:2 (v:v) as mobile phase to yield **7** (19.0 mg) in the form of yellow crystals, mp 283.4-285.4 °C, [α]_D²⁰ +26.1 (c 0.1, CHCl₃) {lit. mp 282-285 °C, [α]_D²⁵ +12.3° (c 0.1, CHCl₃)}.³² F_{6D} (195.8 mg, 3:7 hexane/CH₂Cl₂), was rechromatographed on silica column by elution with hexane, hexane/CH₂Cl₂ (9:1, 8:2, 7:3, 1:1), CH₂Cl₂ and MeOH to yield **9** (47.3 mg) in the form of yellow crystals, mp 78.9-79.9 °C, [α]_D²⁰ +30.2° (c 0.1, CHCl₃) {lit. mp 106-107 °C and [α]_D²⁵ +12.3° (c 0.1, CHCl₃)}.³³ Compound **5** (4.6 mg) was obtained as a yellow resin, [α]_D²⁰ +15.7° (c 0.095, CHCl₃) {lit. [α]_D²⁵ +140.8° (c 0.1, CHCl₃)}.³² from F_{7D} (33.7 mg, 3:7 hexane/CH₂Cl₂), after successive recrystallization with CH₂Cl₂/MeOH, (1:1). F_{8D} (479.7 mg, CH₂Cl₂) was chromatographed on silica column with CH₂Cl₂:MeOH (98:2, 95:5 and 1:1) to give 22 sub-fractions (10 mL each), that were pooled to 6 sub-fractions (F_{8D1}-F_{8D6}), after TLC analysis. F_{8D5} was subjected to HPLC analysis, using hexane/EtOAc, 8:2 (v:v) as mobile phase to yield compounds **1** (4.0 mg), **2** (3.2 mg) and **3** (3.7 mg).

11,12,15-trihydroxy-8,11,13-abietatrien-7-one (**1**)

Yellowish resin; [α]_D²⁰ +17.13° (c 0.0975, CHCl₃); IR ν_{\max} /cm⁻¹ 3385, 2927, 2865, 1726, 1669, 1564, 1459, 1311; HR-ESI-MS m/z 333.2062 [M + H]⁺, (calc. for C₂₀H₂₉O₄, 333.2060); ¹H and ¹³C NMR spectral data, see Table 1.

6 α ,11,12,15-tetrahydroxy-8,11,13-abietatrien-7-one (**2**)

Yellowish resin; [α]_D²⁰ -18.15° (c 0.074, CHCl₃); IR ν_{\max} /cm⁻¹ 3436, 2928, 2869, 1720, 1675, 1611, 1566, 1463, 1372, 1309, 1298, 1128; HR-ESI-MS m/z 349.2007 [M + H]⁺ (calc. for C₂₀H₂₉O₅, 349.2010); ¹H and ¹³C NMR spectral data, see Table 1.

11,12,16-trihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one (**3**)

Yellow resin; [α]_D²⁰ +57.92° (c 0.08, CHCl₃); IR, ν_{\max} /cm⁻¹ 3401, 2927, 2861, 1722, 1671, 1609, 1564, 1461, 1368, 1316; HR-ESI-MS m/z 333.2064 [M + H]⁺ (calc. for C₂₀H₂₈O₄, 333.2060); ¹H and ¹³C NMR spectral data, see Table 1.

(16S)-12,16-epoxy-11,14-dihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one (**4**)

Yellow solid; mp 195.7-197.7 °C; [α]_D²⁰ +26.79° (c 0.24, CHCl₃); IR ν_{\max} /cm⁻¹ 3382, 2926, 2865, 1618, 1459, 1352, 1253, 1016, 807; HR-ESI-MS m/z 331.1889 [M + H]⁺ (calc. for C₂₀H₂₇O₄, 331.1909); ¹H and ¹³C NMR spectral data, see Table 1.

Results and Discussion

The EtOH extract from roots of *Hyptis crassifolia* Mart. ex Benth. was fractionated by silica gel column chromatography after elution with pure or binary mixtures of hexane, CH₂Cl₂, EtOAc and MeOH. The hexane-CH₂Cl₂ (1:1) and CH₂Cl₂ fractions were subjected to various chromatographic procedures leading to the isolation of nine compounds (**1-9**, Figure 1), whose structures were elucidated by spectroscopic methods, such as IR, high resolution mass spectrometry (HRMS) and particularly, ¹H and ¹³C NMR (1D and 2D).

Compound **1** was obtained as a yellowish resin. The IR spectrum exhibited absorption bands for hydroxyl group at 3385 cm⁻¹, Csp³-H groups at 2927 and 2865 cm⁻¹, skeletal bands of benzene ring at 1609 and 1564 cm⁻¹, conjugated C=O at 1669 cm⁻¹ and C-O of phenol and alcohols at 1254 and 1145 cm⁻¹, respectively. The molecular formula C₂₀H₂₈O₄ was determined by HR-ESI-MS, based on the quasi-molecular ion at m/z 333.2062 [M + H]⁺ (calcd. for C₂₀H₂₉O₄ m/z 333.2060). The CPD and DEPT 135° ¹³C NMR spectra displayed 20 and 11 signals, respectively, one from a conjugated aryl ketone carbonyl (δ_C 199.4), six sp² carbons of a benzene ring (δ_C 116.5-147.6), one non-hydrogenated sp³ carbon bearing an oxygen (δ_C 76.9), and 12 non-functionalized sp³ carbons (δ_C 18.1-50.5; two quaternary, one methine, four methylenes and five methyls) (Table 1). The ¹H NMR spectrum showed five characteristic singlets of methyl groups attached to non hydrogenated carbons at δ_H 0.95 (Me-18), 0.98 (Me-19), 1.40 (Me-20), 1.68 (Me-17) and 1.70 (Me-16). A signal at δ_H 7.49 (H-14) was ascribed to one proton of a pentasubstituted benzene ring. In addition, a pair of double doublets at δ_H 2.53 (J 17.0, 14.4 Hz, H-6b) and 2.63 (J 17.0, 2.8 Hz, H-6a) were observed, corresponding to one methylene group coupled to a methine at δ_H 1.85 (dd, J 2.8, 14.4 Hz, H-5). The presence of a hydroxyl group at C-15 was accomplished by the HMBC spectrum analysis, showing long-range correlations of the methyl groups at δ_H 1.70 (Me-16) and 1.68 (Me-17) with the oxygenated carbon at δ_C 76.9 (C-15, ² J) (Figure 2). Furthermore, the pattern of substitution of the pentasubstituted benzene ring

Table 1. ¹H and ¹³C NMR data (δ in ppm, *J* in Hz) for compounds **1-5**

C	1^b		2^b		3^b		4^a		5^a	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.4	3.24 (bd, <i>J</i> 13.5 Hz) 1.44 (m)	36.6	3.21 (bd, <i>J</i> 12.8 Hz) 1.41 (m)	36.4	3.29 (bd, <i>J</i> 13.3 Hz) 1.44 (dd, <i>J</i> 3.6, 13.3 Hz)	37.8	3.47 (bd, <i>J</i> 14.3 Hz) 1.39 (overlap)	38.1	3.44 (bd, <i>J</i> 14.0 Hz) 1.34 (m, 1H)
2	19.2	1.77 (td, <i>J</i> 3.2, 6.4 Hz) 1.60 (m)	19.1	1.70 (overlap) 1.55 (m)	19.2	1.80 (td, <i>J</i> 3.6, 7.0 Hz) 1.60 (td, <i>J</i> 3.6, 7.0 Hz)	20.2	1.55 (bd, <i>J</i> 14.2 Hz) 1.79 (m)	20.3	1.76 (dd, <i>J</i> 2.8, 14.2 Hz) 1.56 (dt, <i>J</i> 3.6, 14.2 Hz)
3	41.5	1.50 (bd, <i>J</i> 13.5 Hz) 1.30 (m)	42.6	1.57 (m) 1.36 (m)	41.5	1.50 (bd, <i>J</i> 13.3 Hz) 1.32 (m)	42.5	1.48 (overlap) 1.33 (m)	42.6	1.50 (bd, <i>J</i> 12.6 Hz) 1.29 (dd, <i>J</i> 4.0, 14.0 Hz)
4	33.7	–	34.3	–	33.4	–	34.5	–	34.5	–
5	50.5	1.85 (dd, <i>J</i> 2.8, 14.4 Hz)	55.6	1.81 (d, <i>J</i> 13.5 Hz)	50.6	1.88 (dd, <i>J</i> 2.9, 14.3 Hz)	51.9	1.75 (dd, <i>J</i> 2.5, 14.7 Hz)	51.7	1.76 (dd, <i>J</i> 2.7, 14.4 Hz)
6	35.8	2.63 (dd, <i>J</i> 2.8, 17.0 Hz) 2.53 (dd, <i>J</i> 14.4, 17.0 Hz)	73.4	4.60 (d, <i>J</i> 13.5 Hz)	35.8	2.63 (dd, <i>J</i> 1.9, 14.8 Hz) 2.54 (dd, <i>J</i> 14.8, 17.0 Hz)	36.4	2.63 (d, <i>J</i> 14.7 Hz) 2.51 (dd, <i>J</i> 2.0, 16.9 Hz)	36.5	2.66 (dd, <i>J</i> 2.7, 16.0 Hz) 2.53 (dd, <i>J</i> 2.7, 16.0 Hz)
7	199.4	–	200.4	–	199.7	–	206.4	–	206.3	–
8	124.6	–	121.6	–	125.3	–	111.3	–	109.8	–
9	139.4	–	138.9	–	139.2	–	142.3	–	138.5	–
10	40.3	–	41.4	–	40.3	–	42.2	–	41.7	–
11	142.8	–	142.9	–	143.4	–	133.6	–	137.4	–
12	147.6	–	148.3	–	148.1	–	158.9	–	159.2	–
13	128.6	–	129.1	–	122.9	–	112.2	–	112.1	–
14	116.5	7.49 (s)	116.7	7.44 (s)	122.4	7.43 (s)	156.6	–	155.4	–
15	76.9	–	76.8	–	40.2	2.90 (dd, <i>J</i> 1.9, 14.8 Hz) 2.78 (dd, <i>J</i> 7.4, 14.8 Hz)	35.0	3.27 (overlap) 2.75 (dd, <i>J</i> 7.3, 15.3 Hz)	32.6	2.87 (dd, <i>J</i> 3.5, 14.3 Hz) 2.78 (dd, <i>J</i> 7.0, 14.3 Hz)
16	30.6	1.70 (s)	30.6	1.71 (s)	71.3	4.31(m)	83.9	5.08 (bq, <i>J</i> 6.9 Hz)	69.9	4.09 (m)
17	30.8	1.68 (s)	30.8	1.69 (s)	23.5	1.29 (d, <i>J</i> 6.2 Hz)	22.3	1.48 (d, <i>J</i> 6.2 Hz)	22.9	1.17 (d, 6.2)
18	33.4	0.95 (s)	35.9	1.21 (s)	33.7	0.95 (s)	34.5	0.96 (s)	33.8	0.96 (s)
19	21.8	0.98 (s)	22.8	1.25 (s)	21.8	1.00 (s)	22.2	0.99 (s)	22.2	0.99 (s)
20	18.1	1.40 (s)	19.1	1.55 (s)	18.1	1.40 (s)	18.2	1.39 (s)	18.3	1.38 (s)
OH		6.09 (s)		6.14 (s)		6.19 (s)		3.51 (s, 1H)		4.58 (s, 1H)
OH		9.86 (s)		9.94 (s)				4.61 (s, 1H)		5.49 (s, 1H)

^aIn 500/125 MHz, CD₃OD; ^bin 500/125 MHz, CDCl₃.

was determined by correlations of the benzene proton at δ_H 7.49 (H-14) with the oxygenated carbon at δ_C 76.9 (C-15, ³*J*), with the sp² carbons at δ_C 147.6 (C-12, ³*J*) and 139.4 (C-9, ³*J*) and with the carbonyl group at δ_C 199.4 (C-7, ³*J*) (Figure 2). Then, compound **1** was characterized as the 11,12,15-trihydroxy-8,11,13-abietatrien-7-one, a diterpene previously synthesized during the structural determination

of callicarpone by Kawazu *et al.*,³⁴ but that is being reported for the first time in the literature as a new natural abietane.

Compound **2** was also obtained as a yellowish resin. The molecular formula C₂₀H₂₈O₅ was determined by HR-ESI-MS, based on the *quasi*-molecular ion at *m/z* 349.2007 [M + H]⁺ (calcd. for C₂₀H₂₉O₅ *m/z* 349.2010). The IR spectrum showed stretching bands consistent with the

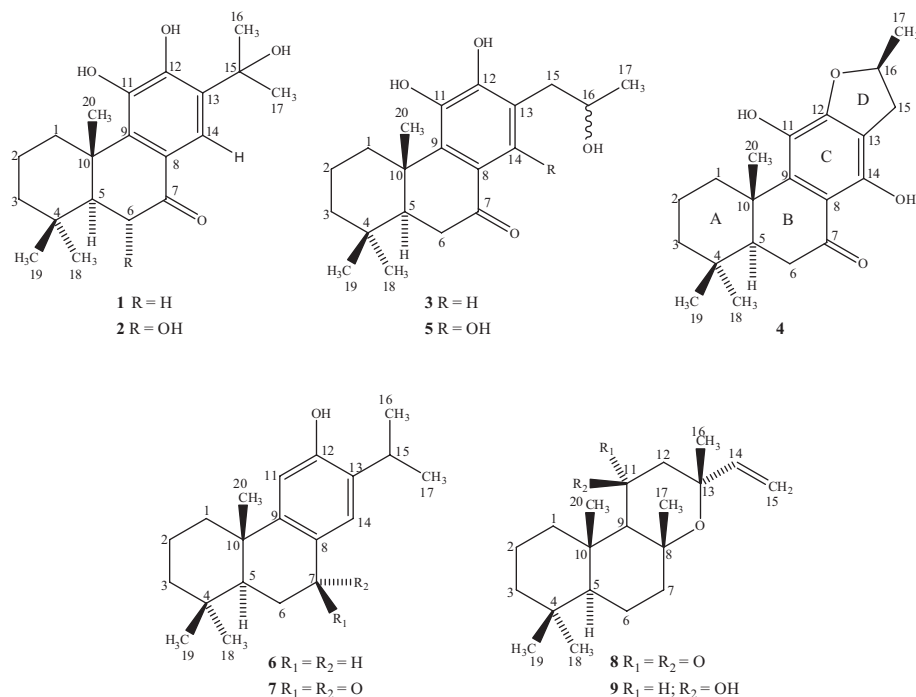


Figure 1. Structures of the diterpenes **1-9** isolated from *H. crassifolia*.

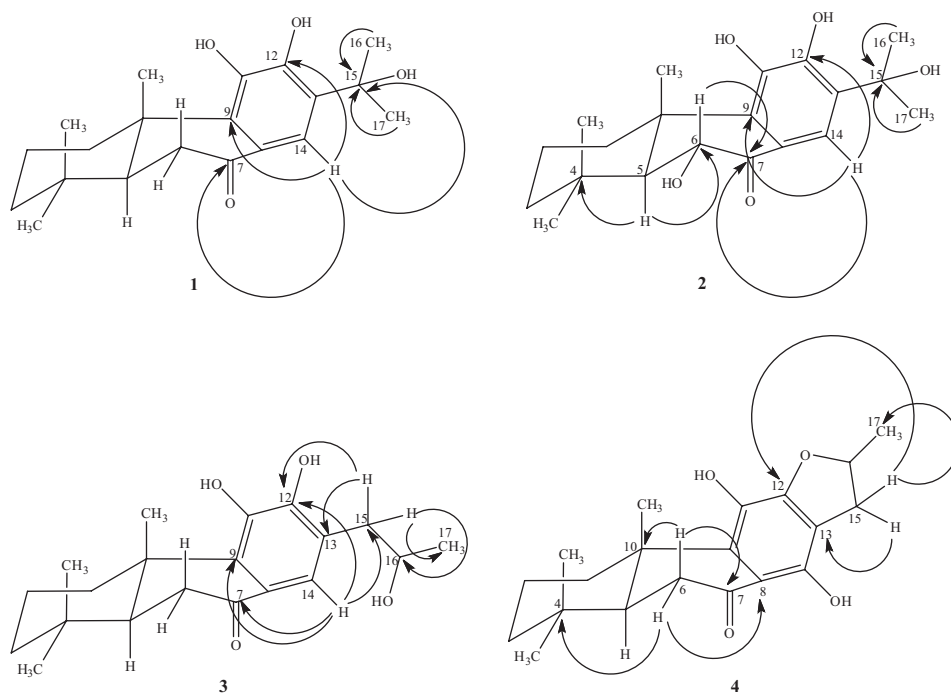


Figure 2. Key HMBC correlations observed for the compounds **1-4**.

presence of hydroxyl groups at 3436 cm^{-1} and stretching bands of $\text{Csp}^3\text{-H}$ groups at 2928 and 2869 cm^{-1} , and as for compound **1**, a conjugated aryl ketone group at 1675 , 1611 and 1566 cm^{-1} . Stretching bands at 1258 and 1080 cm^{-1} were consistent with the presence of C–O group of phenol and alcohols, respectively. Comparative analysis of ^1H and ^{13}C NMR data of the compounds **1** and **2** revealed several

similarities. The major differences in the ^{13}C NMR spectrum were the disappearance of a methylene at $\delta_{\text{C}} 35.8$ for **1** and the appearance of an oxymethine at $\delta_{\text{C}} 73.4$ for **2**. The deshielding of C-5 ($\delta_{\text{C}} 50.5$ in **1**) to $\delta_{\text{C}} 55.6$ and the shielding of C-8 ($\delta_{\text{C}} 124.6$ in **1**) to $\delta_{\text{C}} 121.6$ are in accordance with β and γ effects, respectively, of the hydroxyl positioned at C-6. The stereochemistry of the hydroxyl at C-6 was

assigned as α -equatorial on the basis of the coupling constants of H-5 [δ_{H} 1.81 (d, J 13.5 Hz)] and H-6 [δ_{H} 4.60 (d, J 13.5 Hz)], a typical axial-axial hydrogen coupling of cyclohexane rings. The changes in the ^1H NMR spectrum of **2**, relatively to **1**, are all in agreement with the above discussion. The axial oxymethine hydrogen attached to the α -carbon to the carbonyl appeared at δ_{H} 4.60 (d, J 13.5 Hz), while all methyls attached to quaternary carbons underwent a light deshielding. Accordingly with the afore mentioned spectral data, the structure of **2** was characterized as the new diterpene 6 α ,11,12,15-tetrahydroxy-8,11,13-abietatrien-7-one.

Compound **3** was obtained as a yellow resin. The molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_4$ was determined by HR-ESI-MS, based on the *quasi*-molecular ion at m/z 333.2064 [$\text{M} + \text{H}$] $^+$ (calcd. for $\text{C}_{20}\text{H}_{29}\text{O}_4$ m/z 333.2060). The NMR data of compound **3** showed remarkable similarities with those of compound **1** (see Table 1). The major differences account for an oxymethine at C-16 (δ_{C} 71.3; δ_{H} 4.31 m) and an extra diastereotopic methylene at C-15 [δ_{C} 40.2; δ_{H} 2.90 (dd, J 1.9, 14.8 Hz, H-15a) and δ_{H} 2.78 (dd, J 7.4, 14.8 Hz, H-15b)]. The singlets at δ_{H} 1.70 (Me-16) and 1.68 (Me-17) correspondent to the geminal methyls of the benzene side chain of **1** are missing, while a doublet for a primary methyl C-17 [δ_{C} 23.5; δ_{H} 1.29 (d, J 6.2 Hz)] appears on the ^1H NMR. This information is in agreement with a rearrangement of the aromatic side chain of compound **1**. The ^{13}C NMR data is totally in accordance with the suggested change, appearing the extra methylene (C-15) at δ_{C} 40.2, the oxymethine (C-16) at δ_{C} 71.3, and the methyl (C-17) at δ_{C} 23.5. The other expected changes were the shielding of C-13 (δ_{C} 122.9) due to the replacement of the deshielding β -hydroxy and β -methyl effect on compound **1**, for the shielding γ -effect of both groups on compound **3**. The release of the crowding steric hindrance of the branched side chain on compound **1** through the rearrangement for a normal chain on compound **3** also affects the chemical shift of C-14 (δ_{C} 122.4), now with a remarkable deshielding effect ($\Delta\delta_{\text{C}} = 5.9$). The spectral data discussed above are consistent with a 17(15 \rightarrow 16)-*abeo*-abietane diterpenoid.^{32,35} The skeleton of **3** was confirmed through a detailed analysis of the HMBC spectrum by long-range correlations of the aromatic proton at δ_{H} 7.43 (H-14) with the carbons at δ_{C} 148.1 (C-12, 3J), 139.2 (C-9, 3J) and 40.2 (C-15, 3J), and with the carbonyl carbon in δ_{C} 199.7 (C-7, 3J) (Figure 2). Thus, compound **3** was identified as a new 17(15 \rightarrow 16)-*abeo*-abietane diterpene, the 11,12,16-trihydroxy-17(15 \rightarrow 16)-*abeo*-abieta-8,11,13-trien-7-one.

Compound **4** [$[\alpha]_{\text{D}}^{20} +26.79^\circ$ (c 0.24, CHCl_3)] was isolated as a yellow solid (mp 195.7-197.7 $^\circ\text{C}$). The molecular

formula, $\text{C}_{20}\text{H}_{26}\text{O}_4$, was established after HR-ESI-MS analysis based on the *quasi*-molecular ion at m/z 331.1889 [$\text{M} + \text{H}$] $^+$ (calcd. $\text{C}_{20}\text{H}_{27}\text{O}_4$ m/z 331.1909). Compound **5**, already known, showed very similar spectroscopic data to those of compound **4** (see Table 1). Its HR-ESI-MS *quasi*-molecular ion at 347.1879 [$\text{M} - \text{H}$] $^-$, 18 Da higher than that of compound **4**, suggested that compound **4** could be a dehydrated derivative. The IR spectrum of **4** exhibited absorption bands for hydroxyl groups at 3382 cm^{-1} and a conjugated ketone carbonyl group at 1639 cm^{-1} . The ^{13}C CPD NMR spectrum exhibited 20 signals, that after the ^1H , ^{13}C -HSQC spectrum analysis allowed to determine the presence of an aryl ketone carbonyl, chelated to the hydroxyl at C-14, at δ_{C} 206.4, six sp^2 carbons at the region of δ_{C} 111.3-158.9, one very deshielded oxymethine carbon at δ_{C} 83.9, and 12 sp^3 saturated carbons (δ_{C} 18.2-51.9) (Table 1). Analysis of the ^1H NMR spectrum did not show any aromatic proton, but three singlets of methyl groups attached to quaternary carbons at δ_{H} 0.96 (Me-18), 0.99 (Me-19) and 1.39 (Me-20), and a methyl doublet at δ_{H} 1.48 (d, J 6.2 Hz, Me-17). In addition, an oxymethine proton at δ_{H} 5.08 (bq, J 6.9 Hz, H-16) and a diastereotopic methylene at δ_{H} 3.27 (H-15a); 2.75 (dd, J 15.3, 7.3 Hz, H-15b) have been observed. The main difference was the deshielding of C-16 (δ_{C} 69.9 in **5**) to δ_{C} 83.9 due the formation of an α -methyl-dihydrofuran ring condensed with the fully substituted benzene ring at the positions C-12 and C-13. The appearance of a chelated hydroxy at δ_{H} 13.44 in the ^1H NMR spectrum (CDCl_3) of compound **4** (see Supplementary Information (SI) section) evidenced that the ring closure has been done through the C-12 hydroxy. The substitution pattern of the C aromatic ring was definitively established from the HMBC analysis by long-range correlations of the methylene protons at δ_{H} 3.27 (H-15a) and 2.75 (H-15b) with the carbons at δ_{C} 158.9 (C-12, 3J), 112.2 (C-13, 2J) and 22.3 (C-17, 3J). In addition, the correlations of the methylene protons at δ_{H} 2.63-2.51 (H-6) with the carbons at δ_{C} 34.5 (C-4, 3J), 206.4 (C-7, 2J), 111.3 (C-8, 3J) and 42.2 (C-10, 3J) (Figure 2) were also observed. To the C-16 stereogenic center was attributed the same relative stereochemistry as that in either teuvinenone A or E (αH , βMe), taking in consideration the similar chemical shifts and coupling constant values of the 2H-15, H-16 and Me-17 protons,³⁵ as well as the carbon-13 chemical shifts of the dihydrobenzofuran system (C-8 to C-17) (see Table 1). This, undoubtedly, indicated for compound **4** the structure of the new rearranged abietane diterpene, (16*S*)-12,16-*epoxy*-11,14-dihydroxy-17(15 \rightarrow 16)-*abeo*-abieta-8,11,13-trien-7-one. Comparison of the NMR data of **4**, with those published for villosin A, a compound previously isolated from *Teucrium divaricatum* Subsp. *villosum* by

Ulubelen *et al.*²⁹ to which the structure suggested is the same as the one proposed for **4**, did not show a good matching. For instance, the carbonyl chemical shift at δ_c 185.6 is not compatible with the C-7 aryl ketone carbonyl of compound **4** (δ_c 206.4), once other aryl ketones described in the literature have carbon resonances of approximately δ_c 205.0.^{32,36,37} It appears that the carbonyl chemical shift at δ_c 185.6 previously reported,²⁹ is more compatible with a cross conjugated aryl ketone (ca. δ_c 188.0)³⁶ or α -hydroxy cross conjugated aryl ketones (ca. δ_c 183.0).^{29,36} In addition, several other inconsistencies can be pointed out, for example, the ¹³C NMR chemical shifts of C-4 (δ_c 37.2), C-6 (δ_c 29.7), C-8 (δ_c 107.6) and C-10 (δ_c 35.4).²⁹ Thus, the NMR data assignment previously published for villosin A should be revised, or an alternative structure should be considered. According to the analysis described above, the structure of **4** was assigned as (16*S*)-12,16-epoxy-11,14-dihydroxy-17(15→16)-abeo-abieta-8,11,13-trien-7-one.

The remaining compounds were characterized as incanone (**5**),³² ferruginol (**6**),^{30,38} sugiol (**7**),³⁹ 11-oxomanoyloxide (**8**)⁴⁰ and 11 β -hydroxymanooyloxide (**9**),³³ by extensive 1D and 2D NMR spectroscopy analyses and by comparison of the spectral data with those reported in literature.

Conclusions

The unprecedented phytochemical analysis of *Hyptis crassifolia*, a herb widely dispersed through the neighborhood of Mucujê, a small town at Chapada Diamantina, BA, Brazil, has led to the isolation of four abietane diterpenes, two of which are new, **1** and **2**. This is in agreement with the chemotaxonomic profile of the genus *Hyptis* already reported in the literature. On the other hand, rearranged abietanes, two of which are new (**3** and **4**), are being reported for the first time from *Hyptis*. The known labdane diterpenes **8** and **9**, isolated from other genera like *Salvia*³³ and *Kyllinga*,⁴⁰ have not been reported from *Hyptis* previously. In addition, the NMR data of the rearranged abietane **4**, previously identified as villosin A,²⁹ was reassigned.

Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbc.org.br> as PDF file.

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