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Article

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

Karísia S. B. Lima,[a] Maria L. S. Guedes[b] and Edilberto R. Silveira*[,a]

[a]Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, CP 12.200, 60021-940 Fortaleza-CE, Brazil

[b]Departamento de Botânica, Instituto de Biologia, Universidade Federal da Bahia, 40170-290 Salvador-BA, Brazil

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, 6α,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, (16*S*)-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 11β-hidroximanoíla. Os compostos 11,12,16-tri-hidroxi-17(15→16)-*abeo*-abieta-8,11,13-trien-7-ona e 6α,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 (16*S*)-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) ^{1}H e ^{13}C. 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 ^{1}H e ^{13}C, 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, 6α,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, (16*S*)-12,16-*epoxy*-11,14-dihydroxy-17(15→16)-*abeo*-abieta-8,11,13-trien-7-one, incanone, ferruginol, sugiol, 11-oxomanoyl oxide and 11β-hydroxymanoyl oxide. Compounds 11,12,16-trihydroxy-17(15→16)-*abeo*-abieta-8,11,13-trien-7-one and 6α,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 (16*S*)-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 ^{1}H and ^{13}C 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), ^{1}H and ^{13}C 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]

*e-mail: edil@ufc.br

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*-naphtoquinone 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 literature[29] 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. ^{1}H and ^{13}C 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. ^{1}H NMR (300.13 and 500.13 MHz) and ^{13}C 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 (^{1}H NMR) and the central peak of carbon-13 (^{13}C 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 F_{254}, 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 μm 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/CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2/EtOAc (1:1), EtOAc and MeOH as eluents. The hexane/CH_2Cl_2 (1:1) fraction (674.4 mg) was chromatographed on a silica gel column with hexane, hexane/CH_2Cl_2 (9:1, 7:3, 1:1), CH_2Cl_2 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/CH_2Cl_2) was separated through HPLC using a 9:1 hexane/EtOAc as eluent (v/v), with an injection volume of 200 μL, under isocratic conditions to yield compound **6** (6.0 mg), $[\alpha]_D^{20}$ +25.7 (c 0.2, $CHCl_3$) {lit. $[\alpha]_D^{20}$ +42.9° (c 0.2, $CHCl_3$)}.[30] F_{6HD} (60.2 mg, hexane/CH_2Cl_2 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° (c 0.2, $CHCl_3$) {lit. mp 96-97 °C, $[\alpha]_D^{20}$ −103.2° (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/CH_2Cl_2), after semi-preparative HPLC analysis (mobile phase 98:2 hexane/isopropanol). The CH_2Cl_2 fraction (1.26 g) was chromatographed over silica gel by elution hexane/CH_2Cl_2 (1:1, 3:7), CH_2Cl_2, CH_2Cl_2/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/CH_2Cl_2) 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, $[\alpha]_D^{20}$ +26.1 (c 0.1, $CHCl_3$) {lit. mp 282-285 °C, $[\alpha]_D^{25}$ +12.3° (c 0.1, $CHCl_3$)}.[32] F_{6D} (195.8 mg, 3:7 hexane/CH_2Cl_2), was rechromatographed on silica column by elution with hexane, hexane/CH_2Cl_2 (9:1, 8:2, 7:3, 1:1), CH_2Cl_2 and MeOH to yield **9** (47.3 mg) in the form of yellow crystals, mp 78.9-79.9 °C, $[\alpha]_D^{20}$ +30.2° (c 0.1, $CHCl_3$) {lit. mp 106-107 °C and $[\alpha]_D^{25}$ +12.3° (c 0.1, $CHCl_3$)}.[33] Compound **5** (4.6 mg) was obtained as a yellow resin, $[\alpha]_D^{20}$ +15.7° (c 0,095, $CHCl_3$) {lit. $[\alpha]_D^{25}$ +140.8° (c 0.1, $CHCl_3$)},[32] from F_{7D} (33.7 mg, 3:7 hexane/CH_2Cl_2), after successive recrystallization with CH_2Cl_2/MeOH, (1:1). F_{8D} (479.7 mg, CH_2Cl_2) was chromatographed on silica column with CH_2Cl_2: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; $[\alpha]_D^{20}$ +17.13° (c 0.0975, $CHCl_3$; IR ν_{max}/cm^{-1} 3385, 2927, 2865, 1726, 1669, 1564, 1459, 1311; HR-ESI-MS *m/z* 333.2062 $[M + H]^+$, (calc. for $C_{20}H_{29}O_4$, 333.2060); ^{1}H and ^{13}C NMR spectral data, see Table 1.

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

Yellowish resin; $[\alpha]_D^{20}$ −18.15° (c 0.074, $CHCl_3$); 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 $C_{20}H_{29}O_5$, 349.2010); ^{1}H and ^{13}C NMR spectral data, see Table 1.

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

Yellow resin; $[\alpha]_D^{20}$ +57.92° (c 0.08, $CHCl_3$); 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 $C_{20}H_{28}O_4$, 333.2060); ^{1}H and ^{13}C NMR spectral data, see Table 1.

(16*S*)-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; $[\alpha]_D^{20}$ +26.79° (c 0.24, $CHCl_3$); 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 $C_{20}H_{27}O_4$, 331.1909); ^{1}H and ^{13}C 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, CH_2Cl_2, EtOAc and MeOH. The hexane-CH_2Cl_2 (1:1) and CH_2Cl_2 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, ^{1}H and ^{13}C 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}, Csp^3-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 $C_{20}H_{28}O_4$ was determined by HR-ESI-MS, based on the *quasi*-molecular ion at *m/z* 333.2062 $[M + H]^+$ (calcd. for $C_{20}H_{29}O_4$ *m/z* 333.2060). The CPD and DEPT 135° ^{13}C NMR spectra displayed 20 and 11 signals, respectively, one from a conjugated aryl ketone carbonyl (δ_C 199.4), six sp^2 carbons of a benzene ring (δ_C 116.5-147.6), one non-hidrogenated sp^3 carbon bearing an oxygen (δ_C 76.9), and 12 non-functionalized sp^3 carbons (δ_C 18.1-50.5; two quaternary, one methine, four methylenes and five methyls) (Table 1). The ^{1}H 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, ^2J) (Figure 2). Furthermore, the pattern of substitution of the pentasubstituted benzene ring

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

	1[b]		**2**[b]		**3**[b]		**4**[a]		**5**[a]	
C	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, *J* 13.5 Hz)	36.6	3.21 (bd, *J* 12.8 Hz)	36.4	3.29 (bd, *J* 13.3 Hz)	37.8	3.47 (bd, *J* 14.3 Hz)	38.1	3.44 (bd, *J* 14.0 Hz)
		1.44 (m)		1.41 (m)		1.44 (dd, *J* 3.6, 13.3 Hz)		1.39 (overlap)		1.34 (m, 1H)
2	19.2	1.77 (td, *J* 3.2, 6.4 Hz)	19.1	1.70 (overlap)	19.2	1.80 (td, *J* 3.6, 7.0 Hz)	20.2	1.55 (bd, *J* 14.2 Hz)	20.3	1.76 (dd, *J* 2.8, 14.2 Hz)
		1.60 (m)		1.55 (m)		1.60 (td, *J* 3.6, 7.0 Hz)		1.79 (m)		1.56 (dt, *J* 3.6, 14.2 Hz)
3	41.5	1.50 (bd, *J* 13.5 Hz)	42.6	1.57 (m)	41.5	1.50 (bd, *J* 13.3 Hz)	42.5	1.48 (overlap)	42.6	1.50 (bd, *J* 12.6 Hz)
		1.30 (m)		1.36 (m)		1.32 (m)		1.33 (m)		1.29 (dd, *J* 4.0, 14.0 Hz)
4	33.7	–	34.3	–	33.4	–	34.5	–	34.5	–
5	50.5	1.85 (dd, *J* 2.8, 14.4 Hz)	55.6	1.81 (d, *J* 13.5 Hz)	50.6	1.88 (dd, *J* 2.9, 14.3 Hz)	51.9	1.75 (dd, *J* 2.5, 14.7 Hz)	51.7	1.76 (dd, *J* 2.7, 14.4 Hz)
6	35.8	2.63 (dd, *J* 2.8, 17.0 Hz)	73.4	4.60 (d, *J* 13.5 Hz)	35.8	2.63 (dd, *J* 1.9, 14.8 Hz)	36.4	2.63 (d, *J* 14.7 Hz)	36.5	2.66 (dd, *J* 2.7, 16.0 Hz)
		2.53 (dd, *J* 14.4, 17.0 Hz)				2.54 (dd, *J* 14.8, 17.0 Hz)		2.51 (dd, *J* 2.0, 16.9 Hz)		2.53 (dd, *J* 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, *J* 1.9, 14.8 Hz)	35.0	3.27 (overlap)	32.6	2.87 (dd, *J* 3.5, 14.3 Hz)
						2.78 (dd, *J* 7.4, 14.8 Hz)		2.75 (dd, *J* 7.3, 15.3 Hz)		2.78 (dd, *J* 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, *J* 6.9 Hz)	69.9	4.09 (m)
17	30.8	1.68 (s)	30.8	1.69 (s)	23.5	1.29 (d, *J* 6.2 Hz)	22.3	1.48 (d, *J* 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)

[a]In 500/125 MHz, CD_3OD; [b]in 500/125 MHz, $CDCl_3$.

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, ^3J), with the sp^2 carbons at δ_C 147.6 (C-12, ^3J) and 139.4 (C-9, ^3J) and with the carbonyl group at δ_C 199.4 (C-7, ^3J) (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 $C_{20}H_{28}O_5$ was determined by HR-ESI-MS, based on the *quasi*-molecular ion at *m/z* 349.2007 $[M + H]^+$ (calcd. for $C_{20}H_{29}O_5$ *m/z* 349.2010). The IR spectrum showed stretching bands consistent with the

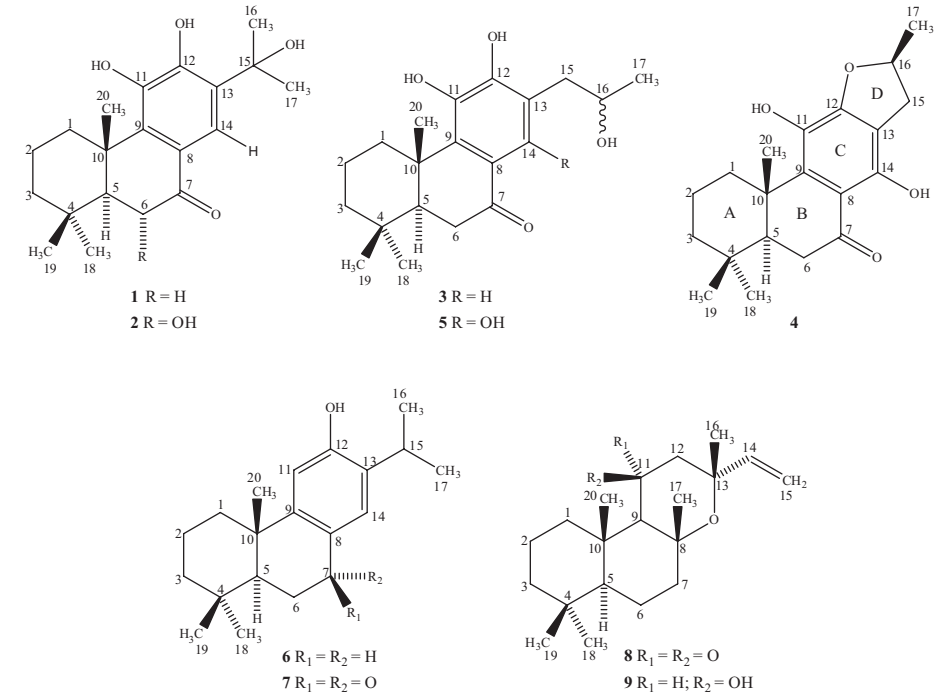

Figure 1. Structures of the diterpenes **1-9** isolated from *H. crassifolia.*

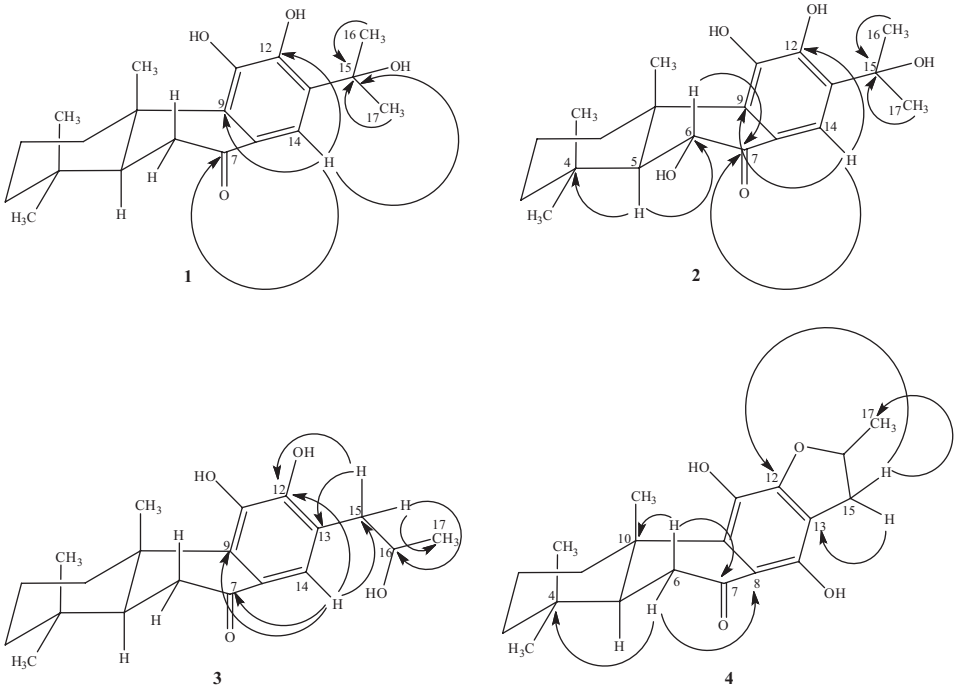

Figure 2. Key HMBC correlations observed for the compounds **1**-**4**.

presence of hydroxyl groups at 3436 cm^{-1} and stretching bands of Csp^3-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 diferences in the ^{13}C NMR spectrum were the disappearance of a methylene at δ_C 35.8 for **1** and the appearance of an oxymethine at δ_C 73.4 for **2**. The deshielding of C-5 (δ_C 50.5 in **1**) to δ_C 55.6 and the shielding of C-8 (δ_C 124.6 in **1**) to δ_C 121.6 are in accordance with β and γ effects, respectivelly, 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 ^{1}H 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 6α,11,12,15-tetrahydroxy-8,11,13-abietatrien-7-one.

Compound **3** was obtained as a yellow resin. The molecular formula $C_{20}H_{28}O_4$ was determined by HR-ESI-MS, based on the *quasi*-molecular ion at *m/z* 333.2064 $[M + H]^+$ (calcd. for $C_{20}H_{29}O_4$ *m/z* 333.2060). The NMR data of compound **3** showed remarkable similarities with those of compound **1** (see Table 1). The major diferences 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 ^{1}H 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 ($\Delta\delta_C = 5.9$). The spectral data discussed above are consistent with a 17(15→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→16)-*abeo*-abietane diterpene, the 11,12,16-trihydroxy-17(15→16)-*abeo*-abieta-8,11,13-trien-7-one.

Compound **4** $[\alpha]_D^{20}$ +26.79º (*c* 0.24, $CHCl_3$) was isolated as a yellow solid (mp 195.7-197.7 °C). The molecular formula, $C_{20}H_{26}O_4$, was established after HR-ESI-MS analysis based on the *quasi*-molecular ion at *m/z* 331.1889 $[M + H]^+$ (calcd. $C_{20}H_{27}O_4$ *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 $[M - H]^-$, 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 ^{1}H,^{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 ^{1}H NMR spectrum did not show any aromatic proton, but three singlets of methyl groups attached to quartenary 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-methyldihydrofuran 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 ^{1}H 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 teuvincenone 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→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 ressonances 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 ^{13}C 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 11β-hydroxymanoyloxide (**9**),[33] by extensive 1D and 2D NMR spectroscopy analyses and by comparison of the spectral data with those reported in literature.

Conclusions

The unprecedent phytochemical analysis of *Hyptis crassifolia*, a herb wildly 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.sbq.org.br as PDF file.

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