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Clerodane and patchoulene terpenes as new constituents from *Baccharis salzmannii* DC

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1. Subject and source

Baccharis salzmannii DC. is a shrub distributed among the states Bahia and Paraná, Brazil. The aerial parts of *B. salzmannii* DC. were collected from Jacobina County (Bahia state, Northeast of Brazil), in March 2009. The specimen was authenticated by Professor Maria Lenise Guedes, from the Instituto de Biologia, Departamento de Botânica, Universidade Federal da Bahia (UFBA), Bahia, Brazil, and a voucher specimen (95175) was deposited at the Herbário Alexandre Leal Costa (ALCB), Universidade Federal da Bahia, Bahia, Brazil.

2. Previous work

Baccharis is large and strictly New World genus belonging to the Asteraceae family, consisting of more than 500 species, about 90% of which are endemic to South America (Verdi et al., 2005). These species are known for the traditional use as medicinal plants and reputed as a rich source of flavonoids (Salcedo et al., 2003; Moreira et al., 2003a, 2003b; Verdi et al., 2004, 2005; Silva et al., 2006; Wollenweber et al., 2006; Guo et al., 2007; Chidiak et al., 2007; Grecco et al., 2010) and

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diterpenes, especially those with clerodane (Li et al., 1997; Cifuente et al., 2002; Hikawczuk et al., 2002; Akaike et al., 2003; Januario et al., 2004; Hayashi et al., 2005; Guo et al., 2006; Hikawczuk et al., 2006; Guo et al., 2007), labdane (Fullas et al., 1994; Cifuente et al., 2000, 2001; Midorikawa et al., 2003; Verdi et al., 2005) and kaurane skeletons (Bohlmann et al., 1981, 1982; Jakupovic et al., 1990; Verdi et al., 2005). Additionally, this genus has been cited to contain coumarins (Bohlmann and Zdero, 1976; Rahalison et al., 1995; Abad and Bermejo, 2007; Kurdelas et al., 2010) and triterpenes (Moreira et al., 2003a, 2003b; Abad and Bermejo, 2007; Verdi et al., 2005; Li et al., 1997; Grecco et al., 2010; Zalewski et al., 2011). Previous phytochemical studies with the aerial parts of *B. salzmannii* (Bohlmann et al., 1981) reports the isolation of naringenin, *ent*-kaur-16-en-19-oic acid and one tiglate derivative.

2.1. Present study

The air-dried and powered leaves of *B. salzmannii* (840 g) were extracted with ethanol to provide the EtOH extract (111.3 g) after removal of the solvent under vacuum. The extract was submitted to partition liquid–liquid with hexane, CH_2Cl_2, EtOAc and MeOH to yield four fractions. The CH_2Cl_2 (80.0 g) fraction was chromatographed on a silica gel column (CC) and eluting with hexane, CH_2Cl_2, EtOAc and MeOH as binary mixtures of increasing polarity to give six fractions. Chromatography of the sub-fraction F-2 (256.0 mg) on a Si gel column using the same eluent system above yielded *ent*-kaur-16-en-19-oic acid (8.0 mg) (Bohlmann et al., 1982). Sub-fraction F-3 was subjected to a silica gel column using CH_2Cl_2 and EtOAc as binary eluent mixture with increasing polarity to afford **2** (28.0 mg) and **3** (5.8 mg). The MeOH fraction was submitted to flash chromatography by elution with hexane and EtOAc, as binary mixtures of increasing polarity, to yield **1** (22.2 mg), naringenin (20.0 mg) (Agrawal, 1989) and apigenin (25.0 mg) (Agrawal, 1989) (Fig. 1).

Compound **1** was obtained as yellow oil, $[\alpha]_D^{20} = -34^\circ$ (*c* 0.001; MeOH). The FT-IR spectrum revealed bands relative to hydroxyl and olefin functional groups at 3375 and 1650 cm^{-1}, respectively. The HRESIMS spectrum gave a molecular ion peak at *m/z* 363.2521 ($[M + Na]^+$, calc. for 363.2511), compatible with the molecular formula $C_{20}H_{36}O_4$. The ^1H NMR spectrum showed signals relative to three methyl groups, two doublets at δ 0.91 (d, $J = 6.6$ Hz, H-16) and 0.84 (d, $J = 6.6$ Hz, H-17), and a singlet at δ 0.77 (s, H-20). In addition, were observed five hydrogens attached to oxygen bearing carbons at δ 4.23 (s, H-18), 4.14 (d, $J = 11.5$, H-19a), 3.78 (d, $J = 11.5$, H-19b), 3.67 (m, H-6) and 3.57 (m, H-15), and an olefinic hydrogen of a trissubstituted double bond at δ 5.83 (br s, H-3).

Comparative analysis of BB and DEPT-^{13}C NMR spectra (Table 1) revealed 20 spectral lines, in agreement with the suggested molecular formula. From this analysis, one can easily deduce the presence of three methyls, nine methylenes, five methines and three non-hydrogenated carbons.

The molecular formula $C_{20}H_{36}O_4$ requires three double bond equivalents. Since a double bond account for one of them, the remaining two unsaturations indicated the bicyclic character of **1**. Based on the above evidences, the structure of a clerodane diterpene does fit the data for compound **1**.

The structure of **1** was unequivocally confirmed through the analysis of the HMQC, HMBC, and ^1H–^1H NOESY spectra. The decaline ring system was characterized by long-range correlations on the HMBC spectrum. This analysis showed the correlations between the olefinic hydrogen at δ 5.83 (H-3) with the methylene at δ 18.9 (C-1), the non-hydrogenated carbon at δ 49.0 (C-5) and the oxymethylene protons at δ 67.7 (C-18). On the other hand, were observed correlations of both oxymethylene at δ 3.78 and 4.14 (2H-19), the oxy-methine at δ 3.67 (H-6) and the methine at δ 1.40 (H-10) with the fully substituted unsaturated carbon at δ 144.0 (C-4). These data indicated the location of the double bond at C3-C4, and the hydroxyls groups at C-18, C-19 and C-6, respectively. In addition, correlations of the protons at δ 1.47 (m, H-13) and δ 0.77 (s, H-20) with the carbon at δ 36.5 (C-11), confirmed a six carbon side chain connected at the C-9. Further, pertinent correlations of the oxy-methylene hydrogens at δ 3.57 (2H-15) with the carbons at δ 31.4 (C-13) and 40.8 (C-14) allowed to confirm the allocation of the remaining hydroxyl group at C-15.

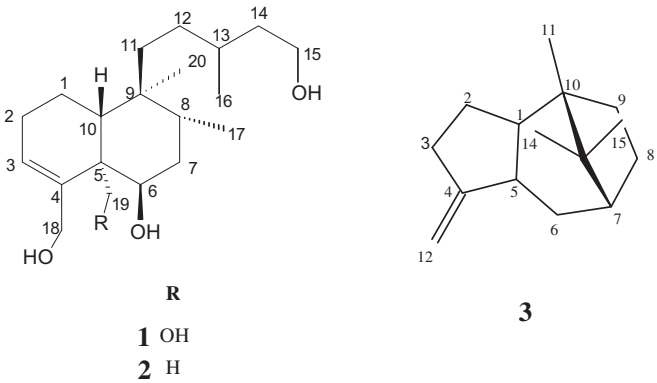

Fig. 1. Structures of compounds **1–3** isolated from *B. salzmannii*

Table 1
^{1}H and ^{13}C NMR spectral data from compounds **1–3**.[a]

Position	**1**		**2**		**3**	
	δc	δ_H	δc	δ_H	δc	δ_H
1	18.9	1.58 (m) 1.78 (m)	19.0	1.64 (m)	69.7	2.34
2	27.5	2.09 (m) 2.20 (td)	27.8	2.07 (m)	27.9	1.40 (m) 1.70 (m)
3	130.5	5.83 (s)	127.3	5.57 (s)	29.9	2.10 (m) 2.35 (m)
4	144.0		147.8		151.7	–
5	49.0		47.0		48.7	2.61 (m)
6	77.0	3.67 (dd, J = 8.0, 7.4)	76.5	3.57 (m)	39.8	1.55 (m) 1.75 (m)
7	38.1	1.63 (m)	37.4	1.36 (m); 1.58 (m)	51.0	1.75 (m)
8	35.9	1.65 (m)	36.0	1.64 (m)	29.9	1.55 (m) 2.25 (m)
9	39.3		39.4		39.2	1.00 (m) 2.00 (m)
10	47.2	1.40 (m)	45.4	1.36 (m)	59.75	–
11	36.7	1.35 (m)	36.7	1.58 (m)	16.9	1.21 (s)
12	30.8	1.05 (m)	30.8	1.08 (m)	112.7	4.86 (s) 4.98 (s)
13	31.4	1.47 (m)	31.4	1.45 (m)	34.1	–
14	40.8	1.32 (m) 1.58 (m)	40.9	1.36 (m)	30.1	1.02 (s)
15	61.2	3.57 (m)	61.2	3.57 (m)	21.6	1.06 (s)
16	20.3	0.91 (d, J = 6.6)	20.4	0.86(d, J = 11.0)		
17	16.0	0.84 (d, J = 6.6)	16.1	0.82 (d, J = 14.7)		
18	67.7	4.23 (s)	66.7	4.04 (d, J = 12.8) 4.17 (d, J = 12.8)		
19	64.3	3.78 (d, J = 11.5); 4.14 (d, J = 11.5)	16.9	1.08 (s)		
20	18.8	0.77 (s)	18.8	0.73 (s)		

[a] ^{1}H and ^{13}C NMR were obtained in MeOD at 500 MHz and 125 MHz, respectively. Chemical shifts were given in ppm: J values are reported in Hz.

The *trans*-fused six-membered of the decaline moiety could be deduced by the key NOE cross-peaks observed between the hydrogens H-6, 2H-19, 3H-17 and 3H-20, that showed these hydrogens oriented in same direction and determined a *cis* relationship between the methyl groups C-17 and C-20. The relative configuration of the hydroxyl group at C-6 was deduced to be β-face on the basis of the coupling constants of H-6 (dd, J = 7.4, 8.0 Hz). These assignments were consistent with 2H-19 and 3H-20 in α-position with an axial orientation, and 3H-17 was in α-position with an equatorial orientation. In addition, correlations observed between H-10, 2H-11, and H-8, revealed these hydrogens on a β-position with an axial orientation. Therefore, the suggested structure for **1** is 6β,15,18,19-tetrahydroxy-*ent*-cleroda-3-ene.

Compound **2** was isolated as a colorless resin, $[\alpha]_D^{20}$: −34° (*c* 0.001, MeOH), showing a molecular ion peak at *m/z* 347.2558 ($[M + Na]^+$, calc. for 347.2562) in the HRESIMS, suggesting $C_{20}H_{34}O_3$ as the molecular formula. The ^{1}H NMR spectrum of compound **2** show very similar signals to those described for **1**. The only light difference was the presence one additional singlet of a methyl group at δ 1.03, and the disappearance of the diastereotopic oxymethylene protons 2H-19.

The comparative analysis of the ^{13}C NMR data of 2 with those observed for compound **1**, revealed signals related to the endocyclic double bond at δ 5.57 (br s, H-3) and the oxymethylene groups at δ 3.57 (m, H-15), 4.04 (d, J = 12.8, H-18a) and 4.17 (d, J = 12.8, H-18b). In addition, the ^{13}C NMR spectrum of **2** presented a shielded signal attributable to methyl group at δ 16.9, in substitution to the signal of the oxymethylene group at δ 64.3 (C-19) observed in **1**.

In the HMBC spectrum the location of the methyl group at C-19 was definitively confirmed by the correlations between the oxymethine at δ 3.57 (H-6) and the methine at δ 1.36 (H-10) with the carbon at δ 16.9. Moreover, the β-orientation of hydroxyl group at C-6 was determined by the NOE correlations observed between the carbinol methine H-6, and the methyl groups H-19 and H-20. From the foregoing evidence, compound **2** was identified as the new 6β,15,18,-trihydroxy-*ent*-cleroda-3-ene.

Compound **3**, was isolated as an optically active colorless oil, and shown to be a sesquiterpene hydrocarbon with the molecular formula $C_{15}H_{24}$ by EI-MS through the molecular ion at *m/z* 204 $[M]^+$. The ^{1}H NMR spectrum of **3** revealed the presence of an exocyclic double bond through the singlets at δ 4.98 (1H, H-12a) and 4.86 (1H, H-12b), and three other intense singlets at δ 1.02 (3H, s, H-14), 1.06 (3H, s, H-15) and 1.21 (3H, s, H-11) assignable to methyl groups attached to quaternary carbons. Chemical shifts and comparative analysis of BB and DEPT – ^{13}C NMR (Table 1) spectra revealed 15 lines in agreement with the suggested molecular formula. From these data one can easily deduce the presence of three methyls, five methylenes, one methylidene, three methines, two quaternaries and one non hydrogenated sp^2 carbon.

The unambiguous assignments of all carbons (Table 1) and hydrogens were possible by the HMQC spectrum analysis. Further evidences of a patchoulene sesquiterpene skeleton were detected by the observed long-range correlations in the

HMBC spectrum, that showed the presence of a *gem*-dimethyl group by the correlations between the methyl hydrogens at δ 1.02 (H-14) with the carbon at δ 21.6 (C−15), and the hydrogens at δ 1.06 (H-15) with the carbon at δ 30.1 (C-14). The same protons also correlated with the carbons at δ 51.0 (C-7) and 59.7 (C-10), while the remaining methyl at δ 1.21 (H-16) showed correlations with the carbons at δ 39.2 (C-9), 69.7 (C-1) and 34.1 (C-13). The analysis of the HMBC spectrum also led to the exact location of the exocyclic double bond at C-4 due to the observed cross-peaks between both diastereotopic methylidene hydrogens at δ 4.86 (H-12a) and 4.98 (H-12b) with the carbons at δ 29.9 (C-3) and 48.7 (C-5), besides the correlations of the hydrogens at δ 1.70 (H-2), 1.75 (H-6) 2.61 (H-5) and 2.35 (H-3) with the carbon at δ 151.7 (C-4). Thus, the above observations led to the deduction of compound **3** as being the new [7,10; 1,5]-patchoul-4(12)-ene.

3. Chemotaxonomic significance

In the present study, three new compounds, two clerodane diterpenes and a new patchoulene-type sesquiterpenoid, together with*ent*-kaur-16-en-19-oic acid, apigenin and naringenin, were obtained from the leaves of *Baccharis salzmanii*.

Diterpenoids and flavonoids have been cited as taxonomically useful chemical markers in Asteraceae, particularly for the subtribe Astereae and for the genus *Baccharis* (Bohlmann et al., 1981). Clerodane are the most widespread diterpenoids and seems to be a constant feature on *Baccharis gaudichaudiana* (Guo et al., 2007, 2006; Akaike et al., 2003), *Baccharis flabellata* (Hikawczuk et al., 2006), *Baccharis trimera* (Januario et al., 2004), *Baccharis sagittalis* (Cifuente et al., 2002) and *Baccharis myrsinites* (Li et al., 1997). The isolation of the new clerodanes 6β,15,18,19-tetrahydroxy-*ent*-cleroda-3-ene (1) and 6β,15,18-trihydroxy-*ent*-cleroda-3-ene (**2**) from *B. salzmannii* are in agreement with these previous results. However, it is worthwhile to comment here upon the structural feature related to open side chain at C-9 on compounds **1** and **2**, since the most common pattern found in clerodane from *Baccharis* contain furan, lactones or γ-butenolides, and this could represent a different evolutionary stage for *B. salzmannii*. In addition, the flavonoid naringenin and apigenin have a wide distribution in the genus *Baccharis*. Naringenin have been cited for *Baccharis ligustrina* (Moreira et al., 2003a,b), *Baccharis retusa* (Grecco et al., 2012), *Baccharis ilinita* (Verdi et al., 2004), *Baccharis conferta* (Weimann et al., 2002), *Baccharis calvescens* (Metawally, 1995), and apigenin was isolated from *B. trimera* (Verdi et al., 2005), *Baccharis tola* (San Martin et al., 1983), *Baccharis salicifolia* (Corral et al., 2012), b. ILINITA (Verdi et al., 2004), *Baccharis pseudotenuifolia* (Moreira et al., 2003a), *B. gaudichaudiana* (Fullas et al., 1994) and *B. retusa* (Grecco et al., 2012). These findings corroborate with the chemotaxonomy of the genus but no relevant conclusion can be drawn from the presence of clerodanes, kaurane and 5,7,4′-trihydroxylated flavonoids owing to their wide distribution in *Baccharis*. On the other hand, the isolation of the [7,10; 1,5]-patchoul-4(12)-ene (**3**), derivative of a polar extract for the first time in the genus, may serve as specific marker of *B. salzmannii*.

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