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Article

Two Clerodane Diterpenes and Flavonoids from *Croton brasiliensis*

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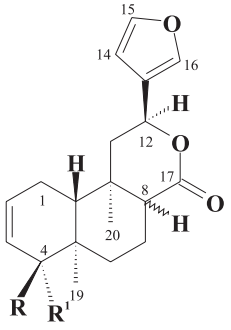
Dois novos diterpenos do tipo clerodano, crotobrasilina A e crotobrasilina B, foram isolados juntamente com quatro 3-metoxiflavonas conhecidas: casticina, penduletina, crisosplenol-D e artemetina, das folhas e caule de *Croton brasiliensis*. A elucidação estrutural destes compostos foi feita com base na análise dos dados espectroscópicos, especialmente RMN, incluindo técnicas bidimensionais (COSY, HMQC, HMBC e NOESY).

Two new clerodane diterpenes, crotobrasilin A and crotobrasilin B, were isolated in addition to four known 3-methoxyflavones: casticin, penduletin, chrysosplenol-D and artemetin from leaves and stems of *Croton brasiliensis*. The structural elucidation of these compounds was made on the basis of spectroscopic data analyses, especially NMR, including 2D techniques (COSY, HMQC, HMBC and NOESY).

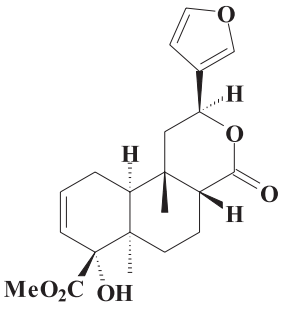
Keywords: *Croton brasiliensis*, clerodane diterpenes, 3-methoxyflavones

Introduction

The genus *Croton* (Euphorbiaceae), which comprises about 700 species, is widely distributed in all regions of Brazil.[1] Many of these species are known in the Brazilian Northeast region as 'capixingui' and 'marmeleiro'[1] and have been used in folk medicine for a large number of applications.[2] Previous phytochemical investigations show that this genus possess alkaloids,[2] flavonoids,[3-6] triterpenoids,[4,7,8] and a large number of diterpenoids.[5,6,9-11] In this paper, we report the isolation and structural elucidation of two new clerodane diterpenes and four known 3-methoxyflavones from leaves and stems of *C. brasiliensis* Mull. Arg.



1 R = CO_2Me, R^1 = OAc 8β, H
2 R = OH, R^1 = CO_2Me, 8α, H
7 R = CO_2Me, R^1 = OH, 8α, H



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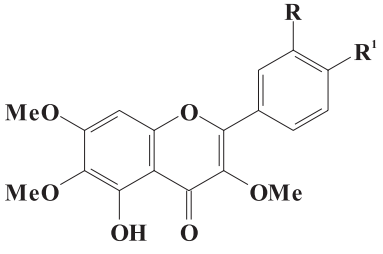
Results and Discussion

The dichloromethane extracts from leaves and stems of *C. brasiliensis* after chromatography fractionation and recrystallizations afforded two clerodane diterpenes (**1**-**2**) in addition to four known 3-methoxyflavones (**3**-**6**).

Compound **1**, isolated as a white amorphous powder, mp 198.5-201.4 °C, by EIMS showed a molecular ion at *m/z* 416. This information, along with NMR data,



3 R = OH, R^1 = OMe
4 R = H, R^1 = OH
5 R = R^1 = OMe
6 R = R^1 = OH

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suggested $C_{23}H_{28}O_7$ as the molecular formula, in accordance with the observed molecular weight. The FT–IR spectrum indicated the presence of ester and lactone carbonyls (1734 cm^{-1}), β-substituted furan ring (1490 and 816 cm^{-1}), and double bond (1665 cm^{-1}). The ^{13}C NMR spectra (CPD, DEPT and HMQC) showed 23 carbon signals (four methyls, four methylenes, eight methines, and seven non-hydrogenated), consistent with substituted diterpene skeleton. The NMR spectra, including ^1H-^1H COSY, NOESY, HMQC and HMBC, showed signals for a *cis*-disubstituted double bond [δ_H 5.81 (m, H-2) and 6.40 (d, *J* 10.3 Hz, H-3); δ_C 131.1 (C-2) and 125.9 (C-3)], a β-substituted furan ring [δ_H 6.21 (br s, H-14), 7.15 (br s, H-16) and 7.16 (br s, H-15); δ_C 109.4 (C-14), 126.7 (C-13), 139.9 (C-16) and 144.0 (C-15)], two angular methyl groups [δ_H 0.80 (s, Me-20) and 1.13 (s, Me-19); δ_C 15.6 (Me-20) and 16.8 (Me-19)], a carbonyl methyl ester group [δ_H 3.49 (s, OMe); δ_C 52.4 (OMe) and 170.6 (C-18)], and signals for an acetyl group [δ_H 1.80 (s); δ_C 21.4 and 170.3], whose correlations observed in the HMBC spectrum (Figure 1), specially for H-6_{ax} (δ 1.37), H-10 (δ 1.94), and Me-19 (δ 1.13) with C-4 (δ 87.8) defined its position in this carbon. Moreover, also were observed signals for sp^3 oxymethine carbon [δ_H 5.06 (dd, *J* 5.1 and 11.6 Hz, H-12); δ_C 71.7 (C-12)], which chemical shifts and multiplicities are characteristic of hydrogen attached to a carbon involved in a δ-lactone moiety [δ_C 171.0 (C-17)]. Correlations observed in the HMBC spectrum (Figure 1) between H-12 (δ 5.06) and C-13 (δ 126.7), C-14 (δ 109.4), and C-16 (δ 139.9) evidenced the relationship between the furan ring and δ-lactone moiety. The remaining NMR data showed characteristic chemical shifts for two methines, four methylenes, and three non-hydrogenated carbons (Table 1). Thus, on the basis in the evidence of the spectral data and comparison with those reported for cordatin (**7**) and *epi*-cordatine (**8**), two β-furan clerodane diterpenes isolated from *Aparisthmium cordatum*[12] and *Burasia madagascarensis*,[13] respectively, structure of a β-furan clerodane diterpene was proposed.

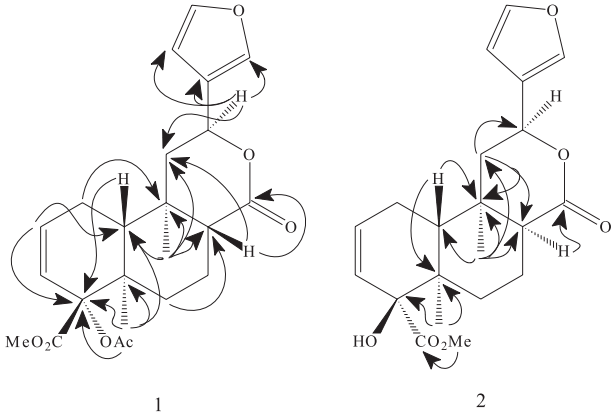

Figure 1. Selected HMBC correlations for **1** and **2**.

NOESY experiment deduced the relative configurations at asymmetric centers (Figure 2). Dipolar-dipolar correlations between H-12 (δ 5.06) and Me-20 (δ 0.80), and this later with Me-19 (δ 1.13), as well as for H-6_{ax} (δ 1.37) and H-8 (δ 1.73) with H-10 (δ 1.94) demonstrated a 1,3-*cis* relationship among these hydrogens and suggested that **1** have A/B and B/C *trans* ring junctions. These relative configurations were also supported by comparison of the NMR data of **1**, exactly when recorded also in $CDCl_3$, with those of **7**, whose structure was determined by X-rays diffraction analysis and possess A/B *trans* and B/C *cis* ring junctions. In particular, compound **1** exhibited significant differences in the chemical shifts of C-8, C-11, and C-17 and showed signals for H-8, H-10 and H-11 at upfield (Table 1). These differences in the chemical shifts these hydrogens can be explained if ring C to adopt a skew-boat conformation, with the ring furan in β-equatorial. Thus, compound **1**, named here crotobrasilin A, was proposed to be the (5R,8R,9R,10R)-4R-acetoxy-15,16-epoxy-*neo*-cleroda-2,13(16),14-trien-17,12S-olide-18-oate methyl.

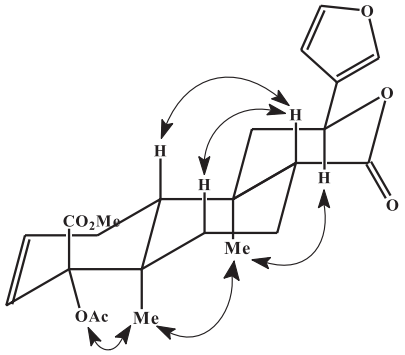

Figure 2. Significant NOESY correlations of **1**.

Compound **2** was isolated as yellowish oil, in a mixture with **1**. Due to the small quantity of the mixture, we have not attempted to separate both compounds in order to avoid any loss of material. However, its NMR data were discernable since furnished different intensities of the signals for both compounds [ratio 2.5:1 (**2**): (**1**)]. So, all assignments of the chemical shifts for **2** were inferred (Table 1). In a similar way that compound **1**, the NMR spectral data of **2** also showed signals for a β-substituted furan ring [δ_H 6.48 (br s, H-14), 7.47 (br s, H-15) and 7.53 (br s, H-16); δ_C 109.9 (C-14), 125.2 (C-13), 141.0 (C-16) and 144.9 (C-15)], a *cis*-disubstituted double bond [δ_H 5.99 (m, H-2) and 5.37 (d, *J* 9.4 Hz, H-

Table 1. NMR data (^{1}H: 500; ^{13}C: 125 MHz, *J* in Hz) for compounds **1** (C_6D_6), **2** ($CDCl_3$), and the models **7** and **8** ($CDCl_3$)

	1[a]		**2**[a]		**7**[12]		**8**[13]	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1_{ax}	23.9	1.77 m	19.1	2.18 m	17.6[b]	2.14 m	25.9	1.96 m
1_{eq}		1.64 m		1.84 (*t*, 7.6)		2.06 m		2.24 m
2	131.1	5.81 m	131.4	5.99 m	123.2	5.90 m	129.6	5.94 (ddd, 9.9; 4.7; 2.7)
3	125.9	6.40 (d, 10.3)	129.7	5.37 (d, 9.4)	129.9	5.35 (d, 10)	125.2	5.62 m
4	87.8		82.1		80.5		78.4	
5	40.7		41.9		40.4		40.9	
6_{ax}	33.9	1.37 (dd, 3.5; 13.4)	29.8	1.46 m	28.3	1.44 m	27.2	1.61 (ddd, 13.3; 13.4; 5.0)
6_{eq}		1.99 m		1.67 m		–		1.32 m
7_{ax}	19.2	1.64 m	24.4	1.87 m	22.9[b]	1.83 m	23.0	1.86 m
7_{eq}		2.28 m		2.18 m		2.10 m		1.97 m
8	51.8	1.73 m	46.2	2.52 m	44.7	2.32 (dd, 5; 12)	44.7	2.61 (dd, 13.4; 6.2)
9	36.6		36.8		35.3		35.0	
10	44.6	1.94 (dd, 5.3; 11.1)	46.1	2.35 (dd, 4.8; 10.8)	44.5	2.49 m	40.6	2.10 (dd, 10.6; 6.4)
11_{ax}	44.5	1.30 (t, 12.5)	50.2	1.84 (t, 7.6)	48.7	1.80 (dd, 5; 12)	41.6	1.82 (dd, 15.4; 12.5)
11_{eq}		1.64 m		2.08 m		2.01 (dd, 3; 12)		2.46 (dd, 15.4; 5.1)
12	71.7	5.06 (dd, 5.1; 11.6)	71.2	5.31 (dd, 5.5; 13.5)	69.7	5.20 (dd, 3; 12)	70.8	5.65 (dd, 12.5; 5.1)
13	126.7		125.2		123.8		124.9	
14	109.4	6.21 br s	109.9	6.48 br s	108.9	6.42 (t, 1.6)	108.4	6.39 (dd, 2.0; 0.7)
15	144.0	7.16 br s	144.9	7.47 br s	143.5	7.48 br s	143.6	7.40 (dd, 2.0; 1.4)
16	139.9	7.15 br s	141.0	7.53 br s	139.5	7.42 (t, 1.6)	139.4	7.43 br s
17	171.0		175.3		175.8		174.5	
18	170.6		177.3		173.7		175.8	
19	16.8	1.13 s	16.1	1.16 s	24.2	1.34 s	20.3	1.25 s
20	15.6	0.80 s	25.8	1.39 s	14.6	1.01 s	33.8	1.11 s
OMe	52.4	3.49 s	54.8	3.85 s	53.2	3.70 s	52.8	3.79 s
MeCO	21.4	1.80 s	–	–	–	–	–	–
MeCO	170.3	–	–	–	–	–	–	–

[a] Assignments made on the basis of DEPT, COSY, NOESY, HMQC, and HMBC for **1** and DEPT, COSY, HMQC, and HMBC for **2**; [b] Signals may be interchangeable.

3); δ_C 131.4 (C-2) and 129.7 (C-3)], two angular methyl groups [δ_H 1.16 (s, Me-19) and 1.39 (s, Me-20); δ_C 16.1 (Me-19) and 25.8 (Me-20)], a carbonyl methyl ester [δ_H 3.85 (s); δ_C 54.8 (OMe) and 177.3 (C-18)], a δ-lactone [δ_H 5.31 (dd, *J* 5.5 and 13.5 Hz); δ_C 71.2 and 175.3], besides signals for two methines, four methylenes, and three non-hydrogenated carbons. The ^{13}C NMR spectra (CPD, DEPT and HMQC) displayed 21 carbon signals assignable to three methyls, four methylenes, eight methines, and five non-hydrogenated carbons. In the general mode, the NMR spectral data of **2** were closely similar to those of **1**, except for the absence of the signals for an acetyl group, which was replaced by a hydroxyl group [**1**: δ_C 87.8 (C-4) and **2**: δ_C 82.1 (C-4)], suggesting that **2** may be the 4-deacetyl-derivative. In fact, the deacetylation to yield the hydroxyl group caused the expected upfield shift of the C-4 ($\Delta\delta$ = -5.7 ppm). Extensive use of NMR techniques, including ^{1}H-^{1}H COSY, HMQC, and HMBC (Figure 1), supported the unambiguous assignments of all ^{1}H and ^{13}C shift values (Table 1).

Although some of the NMR assignments of cordatin (**7**)[12] must be revised, comparison of the ^{13}C NMR data of **2** with those of **7** were very similar, except for the chemical shifts of the C-3 (**2**: δ_C 129.7; **7**: δ_C 123.2, value interchanged in the paper with C-2) and C-18 (**2**: δ_C 177.3; **7**: δ_C 173.7), suggesting that two compounds are epimers at C-4. However, when compared with those of *epi*-cordatine (**8**),[13] which possess A/B and B/C *cis* ring junctions, revealed that the two compounds are not identical since differences in the chemical shifts of the several carbons were observed (Table 1). Therefore, compound **2** was proposed to be the (5R,8S,9R,10R)-4S-hydroxy-15,16-epoxy-*neo*-cleroda-2,13(16),14-trien-17,12S-olide-18-oate methyl, a clerodane diterpene with A/B *trans* and B/C *cis* ring junctions, named here crotobrasilin B.

Several structurally distinct clerodane-type diterpenes have been found in *Croton* species, but to our knowledge, this is the first report of the occurrence of crotobrasilin A and crotobrasilin B from natural sources.

The IR, ^{1}H and ^{13}C NMR spectral data for casticin (**3**),[14,15] peduletin (**4**),[14] artemetin (**5**)[16] and chrysosplenol-D (**6**)[16] were in accordance with those reported in the literature. Several structural types of flavonoids have been

reported from *Croton* species.[3-6] However, all the 3-methoxyflavones related in this work are described for the first time in this genus.

Experimental

Instrumental and chromatography materials

The melting point was measured using an MQAPF-302 apparatus and is uncorrected. Optical rotation was measured on a Perkin-Elmer 341 polarimeter. The NMR spectra were recorded either on a Bruker DRX-500 or Mercury-Varian 200 operating at 500 and 200 MHz, respectively, for ^{1}H and 125 and 50.3 MHz, respectively, for ^{13}C, in C_6D_6 or $CDCl_3$, and DMSO-d_6 solutions with TMS as internal standard. IR spectra were obtained on a FT-IR 1750 Perkin-Elmer spectrometer. EIMS were measured at 70 eV on a HP 5990/5988A spectrometer. Silica gel (70-230 and 230-400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography separations.

Plant material

The leaves and stems of *C. brasiliensis* Mull. Arg. (syn. *C. polyandrus* Spreng) were collected in November 1999, in the Área de Proteção Ambiental de Santa Rita (Mucuri), Marechal Deodoro, Alagoas, Brazil, and was identified by Rosangela P. de Lyra Lemos of the Departamento de Botânica do Instituto do Meio Ambiente do Estado de Alagoas (IMA-AL), where a voucher specimen was deposited (MAC-10752).

Extraction and isolation of the constituents

The air-dried and powdered leaves (1300 g) and stems (900 g) were extracted with acetone followed by 90% EtOH at room temperature. The crude extracts [leaves: acetone (142.8 g); stems: acetone (23.9 g) and EtOH (16.2 g)] were suspended in MeOH-H_2O (3:2) solution and extracted successively with C_6H_{14}, CH_2Cl_2 and EtOAc. The CH_2Cl_2 residue of the acetone extract from leaves (16.7 g) was filtrated on silica gel with C_6H_{14}-CH_2Cl_2 1:1 (7.3 g, A), CH_2Cl_2 (0.9 g, B), CH_2Cl_2-EtOAc 1:1 (2.9 g, C), EtOAc (1.06 g, D) and MeOH (1.51 g, E). Fractions A (7.3 g), B (0.9 g) and C (2.9 g) were chromatographed on silica gel column using C_6H_{14} with increasing amounts of CH_2Cl_2. The subfractions obtained after gel permeation on Sephadex LH-20 (MeOH) and successive recrystallizations from MeOH afforded **1** (33 mg), **3** (21 mg), **4** (40 mg), and **5** (108 mg).

The CH_2Cl_2 residues from stems (5.0 g) were submitted to silica gel filtration with C_6H_{14}-CH_2Cl_2 1:1 (1.2 g, A), CH_2Cl_2 (1.21 g, B), CH_2Cl_2-EtOAc 9:1 (0.94 g, C), EtOAc (1.35 g, D) and MeOH (0.08 g, E). Fraction A (1.2 g), after gel permeation on Sephadex LH-20 (MeOH) and successive recrystallizations from MeOH, afforded a mixture 2.5:1 containing **1** and **2** (16 mg), and **6** (18 mg).

(5R,8R,9R,10R)-4R-Acetoxy-15,16-epoxy-neo-cleroda-2,13(16),14-trien-17,12S-olide-18-oate methyl (*Crotobrasilin A*, ***1***)

White amorphous powder, mp 198.5-201.4 °C; $[\alpha]_D^{20}$ + 25.1° ($CHCl_3$, *c* 0.0052). IR (KBr) ν_{max}/cm^{-1}: 2953, 1734, 1665, 1490, 1450, 1374, 1267, 1134, 1076, 1020, 876, 816, 785; EIMS, 70 eV *m/z* (rel. int.): 416 [M] (2), 401 (M-CH_3, 2), 357 (M-OAc, 10), 374 (M-C_2H_2O, 3), 342 (M-$C_3H_6O_2$, 2), 315 (100), 297 (5), 247 (3), 203 (10), 153 (20), 121 (10), 105 (21), 91 (22); ^{1}H (500 MHz) and ^{13}C (125 MHz, C_6D_6) NMR spectra (see Table 1).

(5R,8S,9R,10R)-4S-Hydroxy-15,16-epoxy-neo-cleroda-2,13(16),14-trien-17,12S-olide-18-oate methyl (*Crotobrasilin B*, ***2***).

Yellowish oil. ^{1}H (500 MHz) and ^{13}C (125 MHz, $CDCl_3$) NMR data are given in Table 1.

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References

1. Schultz, A.R.H.; *Introdução à Botânica Sistemática*, 4ª ed., Editora da Universidade: Porto Alegre, 1984.
2. Farnsworth, N.R.; Blomster, R.N.; Messmer, W.M.; King, J.C.; Persinos, G.J.; Wilkes, J.D.; *Lloydia* **1969**, *32*, 1.
3. Peres, M.T.L.P.; Monache, F.D.; Cruz, A.B.; Pizzolatti, M.G.; Yunes, R.A.; *J. Ethnopharmacol*. **1997**, *56*, 223.
4. Maciel, M.A.M.; Pinto, A.C.; Arruda, A.C.; Pamplona, S.G.S.R.; Vanderlinde, F.A.; Lapa, A.J.; Echevarria, A., Grynberg, N.F.; Colus, I.M.S.; Farias, R.A.F.; Costa, A.M.L.; Rao, V.S.N.; *J. Ethnopharmacol*. **2000**, *70*, 41.

5. Graikou, K.; Aligiannis, N.; Skaltsounis, A.L.; Chinou, I.; Michel, S.; Tillequin, F.; Litaudon, M.; *J. Nat. Prod*. **2004**, *67*, 685.
6. Guadarrama, A.B.A.; Rios, M.Y.; *J. Nat. Prod*. **2004**, *67*, 914.
7. Peres, M.T.L.P.; Monache, F.D.; Pizzolatti, M.G.; Santos, A.R.S.; Beirith, A.; Calixto, J.B.; Yunes, R.A.; *Phytother. Res*. **1998**, *12*, 209.
8. Barbosa, P.R.; Fascio, M.; Martins, D.; Guedes, M.L.S.; Roque, N.F.; *Biochem. Syst*. *Ecol*. **2003**, *31*, 307.
9. McChesney, J.D.; Silveira, E.R.; *Fitoterapia* **1990**, *61*, 172.
10. El Mekkawy, S.; Meselhy, M.R.; Nakamura, N.; Hattori, M.; Kawahata, T.; Otake, T.; *Phytochemistry* **2000**, *53*, 457.
11. Giang, P. M.; Son, P.T.; Lee, J.J.; Otsuka, H.; *Chem. Pharm. Bull*. **2004**, *52*, 879.
12. Dadoun, H.; Mueller, A.H.; Cesário, M.; Guilhem, J.; Pascard, C.; *Phytochemistry* **1987**, *26*, 2108.
13. Mambu, L.; Ramanandraibe, V.; Martin, M.T.; Blond, A.; Grellier, P.; Frappier, F.; *Planta Med*. **2002**, *68*, 377.
14. Wang, Y.; Hamburger, M.; Gueho, J.; Hostettemann, K.; *Phytochemistry* **1989**, *28*, 2323.
15. Sharaf, M.; Mansour, R.M.A.; Saleh, N.A.N.; *Biochem. Syst*. *Ecol.* **1992**, *20*, 443.
16. Agrawal, P.K.; Thakur, R.S.; Bansal, M.C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P.K., ed., Elsevier: New York, 1989, ch. 3.

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