Novel Cassane and Cleistanthane Diterpenes from *Myrospermum frutescens*: Absolute Stereochemistry of the Cassane Diterpene Series

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Four new diterpenes (1−4) were isolated from the leaves of *Myrospermum frutescens* as minor constituents. Chagresnol (1), 6β,18-diacetoxycassan-13,15-diene (2), and chagreslactone (3) possess cassane skeletons, while chagresnone (4) exhibits a cleistanthane skeleton. Molecular structures and their relative stereochemistries were elucidated using NMR spectroscopy in combination with UV, IR, and MS spectral data. Although compound 2 was previously reported as a synthetic product, we report its first isolation as a natural product. Derivative products (10−13) were obtained to test their activities against Chagas's disease. In addition, the absolute stereochemistry of the previously isolated cassane diterpene 5 from *M. frutescens* is presented.

As part of the Panama-ICBG Project, which uses ecological criteria for drug discovery,[1] we previously described five novel cassane diterpenes isolated from *Myrospermum frutescens* (Fabaceae).[2] These have activity against *Trypanosoma cruzi*, the parasite responsible for Chagas's disease. Continuing with our study of *M. frutescens*, here we report the isolation and structural elucidation of four additional new diterpenes, chagresnol (1), 6β,18-diacetoxycassa-13,-15-diene (2), chagreslactone (3), and chagresnone (4), from the EtOAc−MeOH extract of mature leaves. Although we previously obtained diacetate 2 as a synthetic product,[2] we report its first isolation as a natural product here. Cassane diterpenes 1−3 and cleistanthane 4 derive from the pimarane diterpene skeleton by migration of a methyl group (cassane) or an ethyl group (cleistanthane) from C-13 to C-14.[3] These compounds were assayed for activity against extra- and intracellular forms of *T. cruzi* together with the five previously isolated cassanes (5−9) and deacetylated or oxidized derivatives (10−14) of compounds 2, 4, 5, and 6. A comparison of structure and activity is presented here. Determination of the absolute stereochemistry of 5 using the Mosher ester method is also presented.

Results and Discussion

Liquid−liquid partitioning followed by column chromatography of the MeOH−EtOAc crude extract of *M. frutescens* leaves produced a fraction active against *T. cruzi*, which yielded compounds 1 and 3 after successive reversed-phase HPLC and preparative TLC. Chagresnol (1) showed an HRCIMS ion of *m/z* 362.2424, consistent with the formula $C_{22}H_{34}O_4$ and 6 degrees of unsaturation. Inspection of the ^{1}H NMR spectrum for 1 revealed signals closely similar to those for 6β-hydroxy-18-acetoxycassan-13,15-diene (6) isolated previously.[2] Noticeable differences in the
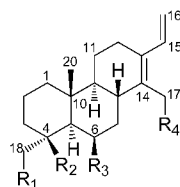
	R_1	R_2	R_3	R_4
1	OAc	CH_3	OH	OH
2	OAc	CH_3	OAc	H
5	OH	CH_3	OH	H
6	OAc	CH_3	OH	H
7	OH	CH_3	H	H
8	OAc	COOH	H	H
10	OH	CH_3	OAc	H
12	OAc	CH_3	=O	H
14	OH	CH_3	=O	H

3

	R_1	R_2
4	OAc	OH
11	OH	OH

9

13
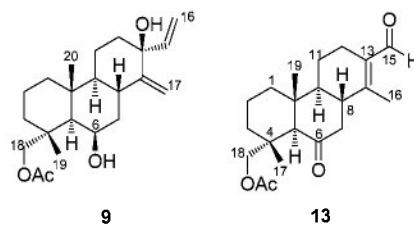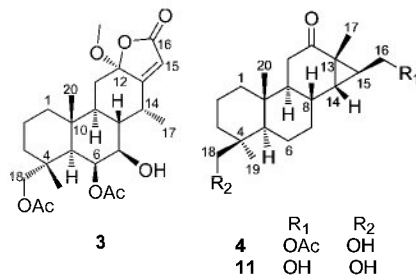
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spectrum for 1 included the absence of a vinyl methyl singlet (δ 1.73, H_3-17 for 6), the appearance of a deshielded

methylene multiplet (δ 4.26), and relatively deshielded terminal double bond signals at δ 6.85 (H-15, dd; δ 6.77 for **6**), 5.08 (H-16a, d; δ 4.94 for **6**), and 5.24 (H-16b, d; δ 5.10 for **6**). Similarly, inspection of the ^{13}C NMR spectrum for **1** showed an additional methylene resonance at δ 59.3 and the absence of a third upfield methyl signal (δ 16.3, C-17 for **6**). These data suggested that **1** possessed a hydroxylated CH$_3$-17, which was consistent with HMBC data and with a molecular mass increase of 16 for **1** compared to **6**. In a 2D NOESY experiment, reciprocal correlations between H-15 and both H-16a and H$_2$-17 (δ 4.26) were observed, as well as a correlation of H-16b (δ 5.24) with H-12b (δ 2.43). These correlations defined a *trans*-conjugated diene in **1**, as in the previously isolated cassanes,[2] which were unfortunately misrepresented as *cis*-conjugated dienes. Hydroxymethylene H-18a (δ 3.72) and H-18b (δ 4.03) correlated with H$_3$-19 (δ 1.25) and α-oriented H-5 (δ 1.23). Methine H-6 (δ 4.31) was also correlated to H-5 (δ 1.23), signifying a β-OH at C-6. Hence chagresnol (**1**) was identified as 6β,17-dihydroxy-18-acetoxycassan-13,-15-diene.

6β,18-Diacetoxycassan-13,15-diene (**2**) was isolated after successive column chromatography, reversed-phase HPLC, and further column chromatography of the hexanes partition fraction obtained from liquid–liquid partitioning (hexanes–10% aqueous MeOH) of the crude leaf extract. The identity of this compound was deduced by comparison of its ^{1}H NMR and optical rotation data with that of chagresnol (**1**) and of the known synthetic compound.[2]

HREIMS data (*m/z* 464.2402) for chagreslactone (**3**) were consistent with a molecular formula of C$_{25}$H$_{36}$O$_8$, implying 8 degrees of unsaturation. The ^{1}H NMR spectrum for **3** showed signals characteristic of a cassane diterpene with an acetylated geminal methyl group at C-4, similar to compounds **1** and **2**. A methoxy methyl singlet (δ 3.20) and a second acetate methyl singlet (δ 2.11) were also evident. This second acetate group could be positioned at C-6 on the basis of an HMBC correlation from H-6 (δ 5.58) to the acetate carbonyl carbon (δ 172.6). In addition, an ABC spin system in **3** was delineated by a double doublet at δ 3.65 (H-7, J = 2, 11 Hz) COSY-coupled to the H-6 doublet (J = 2 Hz) and a multiplet at δ 1.80 (H-8). This was consistent with placement of a hydroxyl substituent at C-7 (δ_C 72.3). Two olefinic (δ 116.3 and 170.8) and three carbonyl (δ 170.1, 171.0, and 172.6) carbon signals in the ^{13}C NMR spectrum for **3** accounted for 4 of the 8 degrees of unsaturation implied by the molecular formula, thus suggesting a tetracyclic metabolite. Quaternary ^{13}C shifts at δ_C 170.8, 170.1, 116.3, and 107.8 were consistent with a fused α,β-butenolide moiety, and both ^{13}C and ^{1}H shifts for ring C and D atoms were closely similar to those reported for 12,-16-epoxy-5α-hydroxy-12α-methoxycassa-13-(15)-en-16-one.[4] The proposed structure was confirmed by HMBC correlations from olefinic H-15 (δ 5.86) to C-13 (δ 170.8), C-14 (δ 32.2), C-16 (δ 170.1), and C-12 (δ 107.8), in combination with correlations from δ 1.80 (H-8) to C-7, C-9, C-14, and C-17, and a three-bond HMBC correlation from the methoxy methyl singlet (δ 3.20) to hemiacetal C-12. The relative stereochemistry of **3** was deduced from 2D NOESY and 1D NOE difference experiments. In 1D NOE difference experiments, irradiation of H-6 (δ 5.58) produced enhancements in H-5 (δ 1.38) and H$_2$-18 (δ 3.74 and 3.93) signals, while irradiation of H-7 (δ 3.65) enhanced H-5 and H-9 (δ 1.53) resonances. These data were confirmed by 2D NOESY data and established β-orientations for both the acetate at C-6 and the lactone ring, with an α-acetoxymethylene (C-18). The methoxy and CH$_3$-17 me-

thyls were assigned α-orientations on the basis of NOESY correlations between these two singlets and also between H-14 (δ 3.38) and H-8 (δ 1.80). Therefore, chagreslactone (**3**) was characterized as 6β,18-diacetoxy-(13),15-ene-7β-hydroxy-12-methoxycassan-12,16-olide. This structure resembles the neocaesalpin[5] and dypteryxic acid[6] type compounds.

Chagresnone (**4**) was assigned a molecular formula of C$_{22}$H$_{34}$O$_4$ on the basis of an HREIMS ion at *m/z* 362.2462 and 22 resonances in the ^{13}C NMR spectrum. Two IR absorptions at 1735 and 1682 cm^{-1} were consistent with ^{13}C resonances for acetyl (δ 170.9) and cycloketone (δ 210.7) moieties, respectively, which accounted for 2 of the 6 degrees of unsaturation implicit in the molecular formula. Thus compound **4** was assigned a tetracyclic carbon skeleton. The ^{1}H NMR spectrum for **4** was similar to those for compounds **1** and **3**. A comparatively shielded pair of H$_2$-18 doublets [δ 3.42 (J = 11 Hz) and 3.73 (1H, d, J = 11 Hz)] could be attributed to the presence of a hydroxymethylene (IR 3462 cm^{-1}) rather than the acetoxymethylene found at C-4 in **1** and **3**. Two comparatively shielded methyl singlets in the spectrum for **4** (δ 0.76, CH$_3$-20 and 0.96, CH$_3$-19) were consistent with the absence of the oxygenated substituent at C-6 in **1** and **3**. An acetate methyl singlet at δ 2.05 and a pair of double doublets at δ 3.82 (1H, J = 6, 12 Hz) and 4.36 (1H, J = 8, 12 Hz) were assigned to an acetoxymethylene moiety at C-15 on the basis of HMBC data. The presence of a cyclopropane ring was indicated by HMBC correlations from H-15 to C-8, C-12, C-14, and C-17 and also from H$_2$-16 to C-13, C-14, and C-15. These correlations, together with a correlation from H$_3$-17 to carbonyl C-12, established the cleistanthane skeleton of **4** and were also consistent with the results of ^{1}H decoupling experiments. The NOESY spectrum for **4** showed correlations from both H$_2$-16 protons to H-14 (δ 1.02), H-15 (δ 1.56), and H$_3$-17. A correlation was also apparent from H$_3$-17 to H-14, but not to H-15. These data supported a *cis* cyclopropyl ring with an β-acetoxymethylene substituent. Remarkably, H$_2$-18 showed a NOESY correlation to H$_3$-20 as well as to H$_3$-19, which in turn showed an intense correlation to H-6b (δ 1.80) and a weaker one to H-6a (δ 1.35). These data suggest that the relative configuration at C-4 is the opposite of that found in cassanes **1**–**3**. Indeed, an axial hydroxymethylene is consistent with the relatively shielded chemical shift of C-18 (δ_C 65.1 compared to δ 73.0 and 72.3 for **1** and **3**, respectively). Thus, chagresnone (**4**) was assigned as 18-hydroxy-16-acetoxy-12-oxocleistanthane.

We previously isolated and reported cassane diterpenes from *M. frutescens*, including **5**–**9**, which are active against *T. cruzi*. We have obtained the absolute configuration at C-6 in **5** using a modified Mosher ester method.[7,8] Both the (*R*)- and (*S*)-methoxyphenyl acetic acid (MPA) esters of cassane **5** were prepared and purified by simple column chromatography. All of the protons of these derivatives were assigned from 2D NMR data (HSQC, HMBC). The $\Delta\delta$ ($\delta_R - \delta_S$) values obtained (Figure 1) were consistent with an *S* configuration at C-6. The other chiral centers in the molecules were determined from their relative configurations.

To investigate structure–activity relationships, we prepared derivatives of 6β,18-diacetoxycassa-13,15-diene (**2**), chagresnone (**4**), and cassane **6**. Alkaline hydrolysis of **2** yielded monoacetate **10**, as evidenced by the absence of a second acetate methyl singlet and the comparatively shielded pair of H$_2$-18 doublets (δ 3.53, J = 11 Hz; 3.14, J = 11 Hz) in the ^{1}H NMR spectrum for **10**, which was
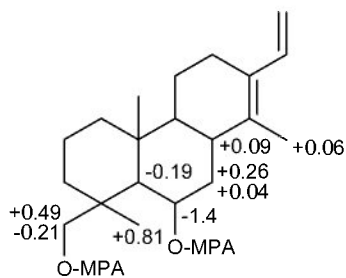
Figure 1. Selected $\Delta\delta^{RS}$ values for MPA derivatives of compound **5**.

otherwise very similar to that for **2**. Alkaline hydrolysis of chagresnone **4** yielded diol **11** (IR 3674, 3403, and 1674 cm^{-1}). Comparison of the ^{1}H NMR spectra for **11** and **4** revealed the absence of an acetate methyl singlet and a comparatively shielded pair of H$_2$-16 double doublets (δ 3.79, $J = 6$, 11 Hz; 3.55 $J = 8$, 11 Hz) in the spectrum for **11**, consistent with the loss of the acetyl group at C-16. Oxidation of compound **6** with Jones reagent yielded a mixture of products from which ketone **12** was isolated. Two carbonyl IR absorptions (1725 and 1710 cm^{-1}) for **12** were consistent with resonances for acetyl (δ 171.0) and cyclohexanone (δ 210.8) carbonyl carbons in the ^{13}C NMR spectrum for **12** and also the absence of an H-6 multiplet (δ 4.27) in its ^{1}H NMR spectrum. Aldehyde **13** was isolated from the same mixture. The ^{1}H and ^{13}C NMR spectra for **13** also showed the presence of a cyclohexanone moiety. However, no olefinic proton signals were evident in the ^{1}H NMR spectrum for **13**. Rather, a sharp singlet at δ 10.15 and a comparatively deshielded double-bond methyl singlet (δ 2.11) indicated the presence of an aldehyde functionality, which was supported by ^{13}C NMR data.

The antitrypanosomal activities of diterpenes **1**−**4** and derivatives **10**−**13** are presented in Table 3. Compounds **6**, **9**, and **12** were more active against the extracellular or infectious form of the parasite (IC$_{50}$ 11, 16, and 17 μM, respectively), while compounds **5** and **7** were more active against the intracellular form of the parasite (IC$_{50}$ 16 and 17 μM, respectively). For the extracellular form, the acetyl group at C-18 appeared to be responsible for the greater activity in **6**, **9**, and **12** relative to compounds with a C-18 hydroxyl group such as **5** (IC$_{50}$ 56 μM), **7** (IC$_{50}$ 48 μM), and **14**[9] (IC$_{50}$ 36 μM). The presence of the acetate group at C-6 in **2** (IC$_{50}$ 59 μM) and **10** (56 μM) may decrease activity due to steric effects. Modifications of the conjugated diene system slightly decreased the activity of **1** and **13** with respect to **6** (IC$_{50}$ 11 μM) and **12**. On the other hand, in the intracellular bioassay, the hydroxyl group on C-18 produced an increase in activity, although modifications at C-6 decreased the activity (**10**, **12**, **13**) relative to the active compounds **5** and **7**. Modifications in the diene system appeared to decrease the cytotoxicity of these compounds to Vero cells. For example, conjugated diene **1** (IC$_{50}$ 156 μM) is more toxic than fully saturated compound **4** (IC$_{50}$ 448 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300 spectrophotometer or a Perkin-Elmer FTIR Spectrum-1000. The NMR spectra were recorded on a Bruker Avance 300 spectrometer with TMS as an internal standard. Irradiation experiments were recorded on a Bruker AMX-500 instrument. HREIMS data were recorded on a UG-Autospec instrument, and the HRCIMS were recorded on a Kratos MS50TC instrument. HPLC was carried out on a Waters LC system, including a

Table 1. ^{1}H NMR Data for Compounds **1**, **3**, and **4** (CDCl$_3$, 300 MHz)

atom no.	δ_H ppm (mult., J/Hz)		
	chagresnol (**1**)	chagreslactone (**3**)	chagresnone (**4**)
1	0.90 m	0.99 m	0.89 m
	1.72 m	1.78 m	1.61 m
2	1.52 m	1.57 m	1.48 m
	1.70 m		
3	1.73 m	1.37 m	0.92 m
			1.30 m
5	1.23 m	1.38 m	1.05 m
6	4.31 m	5.58 d (2)	1.35 m
			1.80 m
7	1.29 m	3.65 dd (2, 11)	2.05 m
	2.24 dt (3, 13)		0.84 m
8	2.67 m	1.80 m	1.67 m
9	0.98 m	1.53 m	1.14 m
11a	1.81 m	1.36 d (3)	1.84 m
11b		2.47 dd (3, 13)	2.20 dd (1.7, 4.1)
12a	2.18 m		
12b	2.43 m		
13			
14		3.38 m	1.02 m
15	6.85 dd (11, 17)	5.86 s	1.56 m
16a	5.08 d (11)		3.82 dd (6, 12)
16b	5.24 d (17)		4.36 dd (8, 12)
17	4.26 m	1.21 d (7.3)	1.22 s
18a	3.72 d (11)	3.74 d (11)	3.42 d (11)
18b	4.03 d (11)	3.93 d (11)	3.73 d (11)
19	1.25 s	1.12 s	0.96 s
20	1.22 s	1.03 s	0.76 s
CH$_3$O-12		3.20 s	
CH$_3$CO-6		2.11 s	
CH$_3$CO-16			2.05 s
CH$_3$CO-18	2.06 s	2.09 s	

Table 2. ^{13}C NMR Data for Compounds **1**, **3**, and **4** (δ_C ppm, CDCl$_3$, 75 MHz)

atom no.	chagresnol (**1**)	chagreslactone (**3**)	chagresnone (**4**)
1	40.6 (CH$_2$)	41.4 (CH$_2$)	38.0 (CH$_2$)
2	18.4 (CH$_2$)	18.0 (CH$_2$)	18.2 (CH$_2$)
3	38.0 (CH$_2$)	37.5 (CH$_2$)	35.5 (CH$_2$)
4	37.4 (C)	37.4 (C)	38.4 (C)
5	51.4 (CH)	48.0 (CH)	55.1 (CH)
6	67.9 (CH)	72.8 (CH)	22.1 (CH$_2$)
7	41.1 (CH$_2$)	72.3 (CH)	35.5 (CH$_2$)
8	33.5 (CH)	42.2 (CH)	36.7 (CH)
9	54.5 (CH)	43.6 (CH)	56.9 (CH)
10	37.4 (C)	37.6 (C)	37.2 (C)
11	21.3 (CH$_2$)	37.2 (CH$_2$)	36.4 (CH$_2$)
12	27.0 (CH$_2$)	107.8 (C)	210.7 (C)
13	134.3 (C)	170.8 (C)	33.4 (C)
14	138.7 (C)	32.2 (CH)	38.5 (CH)
15	134.4 (CH)	116.3 (CH)	33.2 (CH)
16	113.6 (CH$_2$)	170.1 (C)	63.8 (CH$_2$)
17	59.3 (CH$_2$)	11.3 (CH$_3$)	14.3 (CH$_3$)
18	73.0 (CH$_2$)	72.3 (CH$_2$)	65.1 (CH$_2$)
19	19.9 (CH$_3$)	19.6 (CH$_3$)	26.9 (CH$_3$)
20	17.3 (CH$_3$)	17.9 (CH$_3$)	14.7 (CH$_3$)
CH$_3$O-12		51.0 (CH$_3$)	
CH$_3$CO-6		172.6 (C)	
CH$_3$CO-6		21.1 (CH$_3$)	
CH$_3$CO-16			170.9 (C)
CH$_3$CO-16			20.9 (CH$_3$)
CH$_3$CO-18	171.3 (C)	171.0 (C)	
CH$_3$CO-18	21.0 (CH$_3$)	21.7 (CH$_3$)	

600 pump and a 996 photodiode array detector. Melting points were determined using an Electrothermal 9100 apparatus and are uncorrected.

Plant Material. Mature leaves of *M. frutescens* were collected and stored as described previously.[2]

Extraction and Isolation. Fractionation on silica gel 60 (37−75 μm, Geduran) of the MeOH partition derived from liquid−liquid partitioning (hexanes−10% aqueous MeOH) of

Table 3. Compound Activities against *Trypanosoma cruzia*

compound	extracellular IC$_{50}$ (μM)	intracellular IC$_{50}$ (μM)	cytotoxicity[b] IC$_{50}$ (μM)
1	38.8 ± 5.99	76.0 ± 3.84	156 ± 4.64
3	75.0 ± 13.0	ND[c]	225 ± 14.1
4	56.9 ± 1.02	95.5 ± 3.25	448 ± 15.6
10	56.7 ± 5.76	76.5 ± 2.57	178 ± 4.90
11	ND	ND	433 ± 34.1
12	17.7 ± 12.7	79.1 ± 15.9	238 ± 13.4
13	40.4 ± 7.99	61.4 ± 0.12	416 ± 89.2
amphotericin B	1.0	ND	ND
nifurtimox	ND	11.0	ND

[a] Results show the IC$_{50}$ value ± the SD (n = 3). [b] Experiments performed with Vero cells. [c] ND = not determined.

the MeOH−EtOAc extract of leaves of *M. frutescens* yielded four main fractions (1−4). Fraction 2 was again chromatographed on silica gel and eluted with a stepped gradient of hexanes−EtOAc to yield six fractions (2a−2f) as was previously described.[2]

Fraction 2d (516 mg) was subjected to silica gel (37−75 μm) column chromatography and eluted with 1.6 L of CHCl$_3$, 1.0 L of 98:2 CHCl$_3$−acetone, 200 mL of 97:3 CHCl$_3$−acetone, 100 mL of 95:5 acetone−MeOH, 200 mL of 50:50 acetone−MeOH, and 100 mL of MeOH to yield compound **4** (83 mg).

Part of fraction 2 (207 mg) was fractionated by semipreparative reversed-phase HPLC (YMC-Pack ODS-AQ, S5 μm, 12 nm, 150 × 10 mm) using isocratic elution (flow 1.5 mL/min 85:15 MeOH−H$_2$O). Four fractions were obtained (fractions 2g−2j). Fraction 2g (121 mg) was subjected to preparative TLC (Whatman PK5F silica gel 150 A plates) using 75:25 CH$_2$Cl$_2$−EtOAc to yield compound **3** (12 mg). Fraction 2h (60 mg) was subjected to preparative TLC (Whatman PK5F silica gel 150 A plates) and eluted with 60:40 hexanes−EtOAc, yielding compound **1** (12 mg).

The hexanes partition fraction (11.3 g), from liquid−liquid partitioning (hexanes−10% aqueous MeOH) of the crude extract, was chromatographed on silica gel (230−400 mesh) eluted sequentially with 75:25 hexanes−EtOAc, 60:40 hexanes−EtOAc, 1:1 hexanes−EtOAc, 40:60 hexanes−EtOAc, EtOAc, and MeOH. The fractions were combined according to their TLC profiles into nine fractions (1−9). Fraction 2 (2.54 g) was fractionated by isocratic preparative reversed-phase HPLC (Prep Nova Pack HR C$_{18}$, 6 μm, 60 A, 25 × 100 mm; 4.5 mL/min 90:10 MeOH−H$_2$O). Four fractions were obtained (2a−2d). Fraction 2c yielded 905 mg of compound **6**, which was used to prepare derivatives **12** and **13**. Fraction 2d was subjected to silica gel chromatography (7GF) and eluted with 150 mL of 1:1 hexane−CH$_2$Cl$_2$ to yield compounds **7** (38.4 mg) and **2** (24.6 mg).

Chagresnol (1): white solid, [α]^{24}$_D$ −63.8 (c 0.072, CHCl$_3$); IR (KBr) ν_{max} 3445, 2925, 1716, 1384, 1250, 1034 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRCIMS (CH$_4$) m/z (%) 363 [M + 1]$^+$ (6), 362 [M$^+$] (11), 347 (25), 321 (74), 303 (100), 285 (43), 267 (28), 243 (9), 200 (6), 175 (13), 160 (14), 122 (24), 108 (17), 94 (15); HRCIMS (CH$_4$) m/z 362.2424 [M$^+$] (calcd for C$_{22}$H$_{34}$O$_4$, 362.2457).

Chagreslactone (3): [α]^{24}$_D$ −57.1 (c 0.14, CHCl$_3$); IR (KBr) ν_{max} 3446, 2932, 1738, 1654, 1368, 1249, 1058 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LREIMS m/z (%) 464 [M$^+$] (7), 414 (39), 344 (90), 316 (100), 312 (52), 301-(46), 285 (31), 271 (25), 257 (19), 203 (19), 189 (12), 161 (5), 121 (6); HREIMS m/z 464.2402 [M$^+$] (calcd for C$_{25}$H$_{36}$O$_8$, 464.2410).

Chagresnone (4): amorphous powder, mp 138−140°C; [α]^{24}$_D$ +47.8 (c 0.23, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3462, 2928, 2849, 1736, 1682, 1367, 1029 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LREIMS m/z (%) 362 [M$^+$] (5), 302 (100), 271 (18), 229 (3), 192 (4), 149 (9), 121 (6), 108 (12), 81 (2); HREIMS m/z 362.2462 [M$^+$] (calcd for C$_{22}$H$_{34}$O$_4$, 362.2457).

Hydrolysis of 2. Compound **2** (50 mg) was hydrolyzed with 5% KOH in MeOH. After 16 h the mixture was neutralized with 2 N HCl and extracted with CHCl$_3$ and then was washed with water and dried over Na$_2$SO$_4$. Compound **10** (7 mg) was

isolated from the mixture by silica gel column chromatography, eluting with 60:40 hexanes−EtOAc. [α]^{24}$_D$ −56.8 (c 0.22, CHCl$_3$); IR (KBr) ν_{max} 3446, 2994, 1716, 1374, 1257, 1029 cm^{-1}; ^{1}H NMR data (CDCl$_3$, 300 MHz) 6.79 (1H, dd, J = 11, 17 Hz, H-15), 5.46 (1H, d, J = 2.6 Hz, H-6), 5.12 (1H, d, J = 17 Hz, H-16a), 4.98 (1H, d, J = 11 Hz, H-16b), 3.53 (1H, d, J = 11 Hz, H-18a), 3.14 (1H, d, J = 11 Hz, H-18b), 2.07 (3H, s, CH$_3$COO), 1.69 (3H, s, H$_3$-17), 1.47 (1H, m, H-5), 1.17 (3H, s, H$_3$-20), 1.00 (1H, m, H-9), 0.93 (3H, s, H$_3$-19); ^{13}C NMR data (CDCl$_3$, 75 MHz) 170.6 (CH$_3$COO), 135.6 (CH, C-15), 135.4 (C, C-14), 129.4 (C, C-13), 110.9 (CH$_2$, C-16), 71.5 (CH$_2$, C-18), 70.3 (CH, C-6), 54.1 (CH, C-9), 49.2 (CH, C-5), 40.3 (CH$_2$, C-1), 38.1 (C, C-4), 37.5 (CH$_2$, C-3), 37.3 (C, C-10), 36.7 (CH, C-8), 36.7 (CH$_2$, C-7), 26.4 (CH$_2$, C-12), 21.9 (CH$_3$, CH$_3$COO), 21.3 (CH$_2$, C-11), 19.1 (CH$_3$, C-19), 18.3 (CH$_2$, C-2), 16.8 (CH$_3$, C-20), 16.0 (CH$_3$, C-17); LRCIMS (CH$_4$) m/z (%) 347 [M + 1]$^+$ (9), 302 (70), 288 (100), 259 (32), 227 (26), 200 (29), 167 (38), 148 (76), 108 (36), 94 (36); HRCIMS (CH$_4$) m/z 347.2219 [M + 1]$^+$ (calcd for C$_{22}$H$_{34}$O$_3$, 347.2222).

Hydrolysis of 4. Compound **4** (20 mg) was hydrolyzed with 10% KOH in MeOH. After 24 h, the mixture was neutralized with 2 N HCl, extracted with CHCl$_3$, washed with H$_2$O, and dried over Na$_2$SO$_4$. The organic layer gave compound **11** (20 mg): white solid, mp 66−72 °C; [α]^{24}$_D$ +37.0 (c 0.7, CHCl$_3$); IR (KBr) ν_{max} 3674, 3403, 2971, 2922, 1674, 1394, 1066 cm^{-1}; ^{1}H NMR data (300 MHz, CDCl$_3$) δ 3.79 (1H, dd, J = 6, 11 Hz, H-16a), 3.73 (1H, d, J = 11 Hz, H-18a), 3.55 (1H, dd, J = 8, 11 Hz, H-16b), 3.42 (1H, d, J = 11 Hz, H-18b), 1.68 (1H, m, H-8), 1.51 (1H, m, H-16), 1.47 (2H, m, H$_2$-2), 1.23 (3H, s, H$_3$-15), 1.12 (1H, m, H-9), 1.05 (1H, m, H-5), 1.01 (1H, m, H-14), 0.96 (3H, s, H$_3$-19), 0.76 (3H, s, H$_3$-20); ^{13}C NMR data (75 MHz, CDCl$_3$) δ 211.4 (C=O, C-12), 65.1 (CH$_2$, C-18), 62.3 (CH$_2$, C-17), 57.0 (CH, C-9), 55.2 (CH, C-5), 38.4 (C, C-4), 38.3 (CH, C-14), 38.0 (CH$_2$, C-1), 37.3 (CH, C-16), 37.2 (C, C-10), 36.8 (CH, C-8), 36.4 (CH$_2$, C-11), 35.5 (CH$_2$, C-7), 35.5 (CH$_2$, C-3), 33.4 (C, C-13), 26.9 (CH$_3$, C-19), 22.1 (CH$_2$, C-6), 18.2 (CH$_2$, C-2), 14.7 (CH$_3$, C-20), 14.1 (CH$_3$, C-15); LRCIMS (CH$_4$) m/z (%) 321 [M +1]$^+$ (76), 320 [M$^+$] (59), 303 (100), 271 (23), 259 (9), 193 (11), 175 (14), 160 (14), 122 (16), 107 (8), 94 (4); HRCIMS (CH$_4$) m/z 320.2338 [M$^+$] (calcd for C$_{20}$H$_{32}$O$_3$, 320.2351).

Oxidation of 6. Compound **6** (170 mg) was dissolved in acetone (previously distilled over KMnO$_4$) and Jones reagent added in the usual manner. The products were purified by column chromatography on silica gel 60, yielding ketone **12** (14.5 mg): [α]^{24}$_D$ −36.5 (c 0.23, CHCl$_3$); IR (KBr) ν_{max} 3445, 2931, 1725, 1710, 1464, 1380, 1037 cm^{-1}; ^{1}H NMR partial data (CDCl$_3$, 300 MHz) δ 6.78 (1H, dd, J = 11, 17 Hz, H-15), 5.16 (1H, d, J = 17 Hz, H-16a), 5.02 (1H, d, J = 11 Hz, H-16b), 4.05 (1H, d, J = 11 Hz, H-18a), 3.77 (1H, d, J = 11 Hz, H-18b), 1.70 (3H, s, H$_3$-17), 1.23 (3H, s, H$_3$-19), 0.86 (3H, s, H$_3$-20); ^{13}C NMR data (CDCl$_3$, 75 MHz) δ 210.8 (C=O, C-6), 171.0 (MeCO-18), 135.0 (CH, C-15), 134.3 (C, C-14), 129.4 (C, C-13), 112.0 (CH$_2$, C-16), 71.9 (CH$_2$, C-18), 60.2 (CH, C-5), 53.6 (CH, C-9), 47.7 (CH$_2$, C-7), 42.8 (CH, C-8), 41.3 (C, C-10), 38.2 (CH$_2$, C-1), 36.3 (CH$_2$, C-3), 35.3 (C, C-4), 26.4 (CH$_2$, C-12), 21.5 (CH$_2$, C-11), 21.0 (CH$_3$CO-18), 17.8 (CH$_3$, C-19), 17.8 (CH$_2$, C-2), 16.0 (CH$_3$, C-20), 15.5 (CH$_3$, C-17); LREIMS m/z (%) 345 [M + 1]$^+$ (5), 287 (75), 271 (46), 257 (42), 203 (10), 167 (100), 149 (91), 123 (78), 109 (76), 91 (38); HREIMS m/z 345.2048 [M + 1]$^+$ (calcd for C$_{22}$H$_{23}$O$_3$, 345.2073); and aldehyde **13** (7.0 mg): [α]^{24}$_D$ −23.4 (c 0.32, CHCl$_3$); IR (KBr) ν_{max} 3446, 2933, 2361, 1730, 1711, 1380, 1038 cm^{-1}; ^{1}H NMR partial data (CDCl$_3$, 300 MHz) 10.15 (1H, s, CHO), 4.09 (1H, d, J = 11 Hz, H-18a), 3.78 (1H, d, J = 11 Hz, H-18b), 211 (3H, s, H$_3$-16), 2.04 (3H, s, CH$_3$CO-18), 1.25 (3H, s, H$_3$-19), 0.88 (3H, s, H$_3$-20); ^{13}C NMR data (CDCl$_3$, 75 MHz) 209.4 (C=O, C-6), 191.2 (CHO), 170.9 (CH$_3$CO-18),155.4 (C, C-13), 134.4 (C, C-14), 71.9 (CH$_2$, C-18), 60.3 (CH, C-5), 52.9 (CH, C-9), 46.3 (CH$_2$, C-7), 43.6 (CH, C-8), 41.1 (C, C-10), 38.4 (CH$_2$, C-1), 36.1 (CH$_2$, C-3), 35.7 (C, C-4), 23.9 (CH$_2$, C-12), 20.9 (CH$_3$CO-18), 20.8 (CH, C-11), 17.9 (CH$_3$, C-17), 17.7 (CH$_2$, C-2), 16.5 (CH$_3$, C-19), 14.6 (CH$_3$, C-16); LRCIMS (CH$_4$) m/z (%) 347 [M + 1]$^+$ (7), 303 (31), 287 (100), 257 (27), 245 (9), 229 (6), 204 (7), 175 (6), 167 (13), 148 (11), 123 (22), 108 (18), 80 (9); HRCIMS (CH$_4$) m/z 346.2137 [M]$^+$ (calcd for C$_{21}$H$_{30}$O$_4$, 346.2144).

Assignment of Absolute Stereochemistry. The MPA esters of the diol **5** were prepared by treatment with the corresponding (*R*)- and (*S*)-MPA in the presence of DCC and DMAP in CH_2Cl_2. The reaction mixture was filtered to remove the dicyclohexylurea and the ester purified by flash chromatography on silica gel eluting with hexanes—EtOAc. The spectra of the resulting MPA esters were recorded, the signals assigned, and the $\Delta\delta^{RS}$ values calculated (Figure 1). The model designed by the Riguera group was used for configuration assignment of the secondary alcohols.[8]

6*β*,18-Di[(*R*)-methoxyphenyl acetate]cassan-13,15-diene: $[\alpha]^{24}_D$ −87.8 (*c* 1.03, $CHCl_3$); ^{1}H NMR data ($CDCl_3$, 500 MHz) 6.83 (1H, dd, *J* = 11, 17 Hz, H-15) 5.16 (1H, d, *J* = 17 Hz, H-16a), 5.01 (1H, d, *J* = 11 Hz, H-16b), 4.30 (1H, d, *J* = 11 Hz, H-18a), 3.93 (1H, s, H-6), 3.42 (1H, d, *J* = 1 Hz, H-18b), 1.80 (1H, m, H-7a), 1.64 (3H, s, H_3-17), 1.10 (3H, s, H_3-19), 1.04 (3H, s, H_3-20), 0.69 (1H, m, H-5), 0.46 (1H, m, H-7b); ^{13}C NMR data ($CDCl_3$, 125 MHz) 136.4 (C, C-14), 135.4 (CH, C-15), 128.8 (C, C-13), 110.6 (CH_2, C-16), 72.5 (CH_2, C-18), 67.7 (CH, C-6), 53.9 (CH, C-9), 50.0 (CH, C-5), 40.4 (CH_2, C-1), 40.1 (CH_2, C-7), 37.7 (CH_2, C-3), 36.7 (C, C-10), 35.8 (CH, C-8), 32.6 (C, C-4), 26.5 (CH_2, C-12)21.1 (CH_2, C-11), 19.6 (CH_3, C-19), 18.2 (CH_2, C-2), 17.0 (CH_3, C-20), 15.9 (CH_3, C-17); EIMS *m/z* 434 [M − $C_9H_{10}O_3$]+ (10), 268 (34), 253 (14), 239 (11), 183 (8), 121 (100), 105 (12), 91 (14).

6*β*,18-Di[(*S*)-methoxyphenyl acetate]cassan-13,15-diene: $[\alpha]^{24}_D$ −29.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$, 500 MHz) 6.80 (1H, dd, *J* = 11, 17 Hz, H-15), 5.33 (1H, s, H-6), 5.10 (1H, d, *J* = 17 Hz, H-16a), 4.95 (1H, d, *J* = 11 Hz, H-16b), 3.81 (1H, *J* = 11 Hz, H-18a), 3.64 (1H, *J* = 11 Hz, H-18b), 1.58 (3H, s, H_3-17), 1.54 (1H, m, H-7a), 0.92 (3H, s, H_3-20), 0.88 (1H, m, H-5), 0.42 (1H, m, H-7b), 0.28 (3H, s, H_3-19); ^{13}C NMR data ($CDCl_3$, 125 MHz) 136.8 (C, C-14), 135.3 (CH, C-15), 128.8 (C, C-13), 111.1 (CH_2, C-16), 71.2 (CH_2, C-18), 70.1 (CH, C-6), 53.7 (CH, C-9), 48.6 (CH, C-5), 40.5 (CH_2, C-1), 40.2 (CH_2, C-7), 37.1 (CH, C-8), 36.9 (C, C-10), 36.6 (C, C-4), 36.3 (CH_2, C-3), 26.4 (CH_2, C-12), 22.7 (CH_2, C-11), 18.1 (CH_3, C-19), 17.3 (CH_2, C-2), 16.7 (CH_3, C-20), 15.8 (CH_3, C-17); EIMS *m/z* 434 [M − $C_9H_{10}O_3$]+ (37), 268 (90), 185 (11), 132 (8), 121, (100), 105 (8), 91 (9).

***T. cruzi* Bioassay.** The assay is based on inhibition of the parasites by added compound or extract.[2] A *β*-galactosidase-expressing transgenic *T. cruzi* (Tulahuen strain, clone C4) was used. Growth of the intracellular form of *T. cruzi* was determined from the cleavage of chlorophenol red-*β*-D-galactoside (CPRG, 570 nm).

Cytotoxicity Assay. Vero cells adhering to 96-well plates were used to evaluate the toxicity of the compounds purified from *M. frutescens* on the basis of reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide[10] (MTT, Sigma). After treatment with the test compound and 4 h incubation at 37°, cell viability was evaluated in an ELISA reader at 570 nm.

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