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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · NOVEMBER 2011

Impact Factor: 3.8 · DOI: 10.1021/np2002012 · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

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Cembranolides from the Leaves of *Croton gratissimus*

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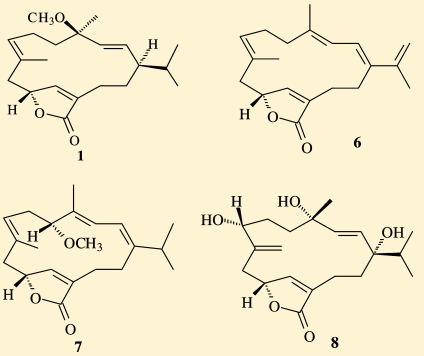
Supporting Information

ABSTRACT: Ten new cembranolides, (−)-(1*R**,4*R**,10*R**)-4-methoxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**1**), (−)-(1*S**,4*R**,10*R**)-1-hydroxy-4-methoxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**2**), (−)-(1*S**,4*S**,10*R**)-1,4-dihydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**3**), (−)-(1*S**,4*S**,10*R**)-1,4-dihydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**4**), (+)-(10*R**)-cembra-1*E*,3*E*,7*E*,11*Z*,16-pentaen-20,10-olide (**5**), (+)-(10*R**)-cembra-1*Z*,3*Z*,7*E*,11*Z*,15-pentaen-20,10-olide (**6**), (+)-(5*R**,10*R**)-5-methoxycembra-1*E*,3*E*,7*E*,11*Z*,15-pentaen-20,10-olide (**7**), (+)-(1*S**,4*S**,7*R**,10*R**)-1,4,7-trihydroxycembra-2*E*,8(19),11*Z*-trien-20,10-olide (**8**), (−)-(1*S**,4*S**,7*S**,10*R**)-1,4,7-trihydroxycembra-2*E*,8(19),11*Z*-trien-20,10-olide (**9**), and (+)-(1*S**,4*R**,8*S**,10*R**)-1,4,8-trihydroxycembra-2*E*,6*E*,11*Z*-trien-20,10-olide (**10**), together with six known compounds, lupeol, 4(15)-eudesmene-1*β*,6*α*-diol, *α*-glutinol, 24-ethylcholesta-4,22-dien-3-one, (+)-(1*R**,10*R**)-cembra-2*E*,4*E*,7*E*,11*Z*-tetraen-20,10-olide, and (+)-(1*R**,4*S**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**4a**), have been isolated from the leaves of *Croton gratissimus*. The acetyl derivatives of **8** and **4a** were evaluated against a chloroquine-sensitive strain of *Plasmodium falciparum* (D10).



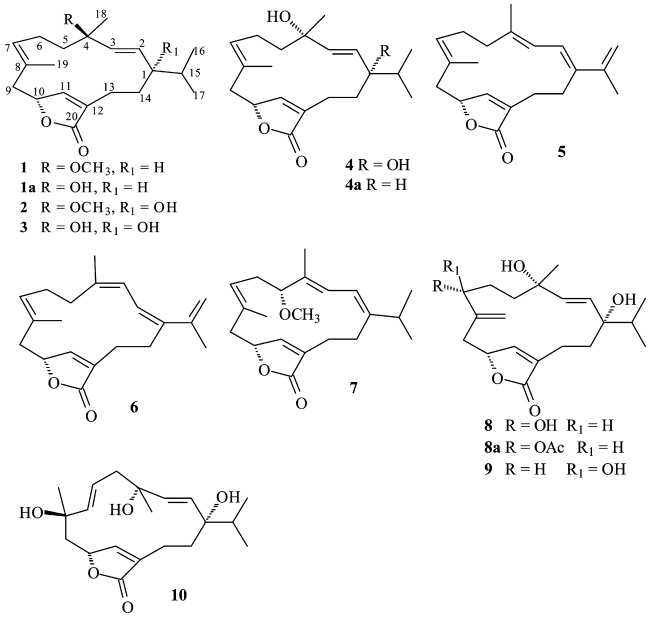
Croton gratissimus Burch. (*C. zambesicus* Müll. Arg.; *C. microbotryus* Pax.) (Euphorbiaceae) grows on stony hillsides as a semideciduous tree species throughout much of the warmer and drier regions from South Africa, northeastwards, to the horn of Africa. The leaves of this plant are ground with goat fat and those of two other *Croton* species, the resulting paste is heated on coals, and the fumes are inhaled as a cure for insomnia.[1] A tea made from the leaves is taken for coughs in Botswana.[2] Watt and Breyer-Brandwijk[3] documented *C. gratissimus* as a remedy for fever, for treating bleeding gums and eye disorders, and as an ingredient used to "smoke" rheumatic patients. In South Africa, the leaves are dried and smoked for influenza, colds, and fevers.[4] The traditional use of this species for treating fevers, intermittent or otherwise, indicates its likely application as an antimalarial, since it is employed as such within the malaria endemic region of southern Africa. In Nigeria, bark infusions of the species (as *C. zambesicus*) are reported to treat malaria.[5] Accordingly, crude dichloromethane extracts of South African leaves of *C. gratissimus* have previously been investigated for antiplasmodial activity in vitro and shown to have an IC_{50} value of 3.5 μg/mL;[6] the constituent(s) responsible were not identified. The stem bark of *C. gratissimus* has been reported to yield four cembranoids, namely, (+)-(1*R**,2*S**,7*S**,8*S**,12*R**)-7,8-epoxy-2,12-cyclocembra-3*E*,10*Z*-dien-20,10-olide, (+)-(1*R**,10*R**)-cembra-2*E*,4*E*,7*E*,11*Z*-tetraen-20,10-olide, (+)-(1*R**,4*S**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**4a**), and (−)-(1*R**,4*R**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide, in addition to lupeol, 4(15)-eudesmene-1*β*,6*α*-diol, and *α*-glutinol.[7]

RESULTS AND DISCUSSION

The combined hexane and methylene chloride extracts (shown to be similar by TLC analysis) and the ethyl acetate extract of the leaves of *C. gratissimus* were separated using repeated column chromatography over silica gel to yield 10 new cembranolides, **1**−**10**, all of which were colorless oils. These were obtained together with six known compounds, eudesm-4(15)-ene-1*β*,6*α*-diol,[8] *α*-glutinol,[9] lupeol,[10] (+)-(1*R**,4*S**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**4a**), and (+)-(1*R**,10*R**)-cembra-2*E*,4*E*,7*E*,11*Z*-tetraen-20,10-olide, which have been isolated previously from the bark of this species,[7] and 24-ethylcholesta-4,22-dien-3-one.[11] NMR analysis showed that purified cembranolides are unstable on standing, but appear to be stable in the unpurified form.

Compound **1** was identified as (−)-(1*R**,4*R**,10*R**)-4-methoxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide. The NMR spectra were very similar to those of (−)-(1*R**,4*R**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**1a**), previously isolated from the bark, for which the relative configuration was deduced by comparison with that of the crystalline

Received: March 31, 2011
Published: October 27, 2011



(+)-(1*R**,2*S**, 7*S**,8*S**,12*R**)-7,8-epoxy-2,12-cyclocembra-3*E*,10*Z*-dien-20,10-olide isolated from the same source.[7] The HRMS indicated a molecular formula of $C_{21}H_{32}O_3$, and the FTIR spectrum gave an absorption band at 1759 cm^{-1}, consistent with an α,β-unsaturated γ-lactone, a functional group present in all cembranolides isolated.[12] The ^{13}C NMR spectrum displayed 21 carbon resonances, including those of a carbonyl carbon (δ_C 174.2, C-20), six olefinic carbons (δ_C 149.6, 135.8, 134.0, 133.5, 131.7, and 129.4), an oxymethine (δ_C 80.3), and five methyl group carbons (δ_C 50.4, 20.7, 22.3, 19.5, and 16.3). The ^1H NMR spectrum showed the presence of four olefinic proton resonances, one at δ_H 6.98 (brd, J = 1.8 Hz) and three overlapping resonances at δ_H 5.40 (m), a methoxy (δ_H 3.15), four methyl group proton resonances at δ_H 1.63 (s), 1.25 (s), 0.91 (d, J = 6.6 Hz), and 0.86 (d, J = 6.6 Hz), one vinylic methyl group, and the presence of an isopropyl group, as in compounds previously isolated from the bark extract.[7] The two methyl group proton doublets (H_3-16, H_3-17) were coupled to a methine proton resonance at δ_H 1.62 (H-15), which was superimposed with the H-1 resonance, confirming the presence of an isopropyl group. The H-1 resonance was coupled to a two olefinic proton resonance at δ_H 5.40 (H-2, H-3) that showed no further coupling. The HMBC spectrum showed correlations of the H-2/H-3 with the C-1 (δ_C 48.9), C-4 (δ_C 77.2), C-5 (δ_C 43.7), and C-18 (δ_C 22.3) resonances. The methoxy proton showed a correlation in the HMBC spectrum with the C-4 resonance, indicating its attachment to C-4, and the C-4 resonance showed correlations with H_3-18 and one of a pair of methylene group proton resonances at δ_H 1.85 (H-5). This proton resonance showed coupling with δ_H 2.35 (m) and 1.63 (m) at C-6, which, in turn, showed coupling with an olefinic proton (H-7, δ_H 5.40). The H-7 protons showed correlations in the HMBC spectrum with the C-6 (δ_C 25.5), C-19 (δ_C 16.3), and C-9 resonances (δ_C 44.8). The magnitude of the ^{13}C NMR chemical shift of a vinylic methyl carbon resonance has been used by Crombie et al.[13] and Olsson et al.[14] to determine the configuration of a methyl-substituted double bond. Lange and Lee reported that a vinylic methyl group carbon resonance value of <20 ppm is indicative of an *E* configuration, whereas a carbon resonance value of >20 ppm is indicative of a *Z* configuration.[15] As the chemical shift of the C-19 methyl carbon occurred at δ_C 16.3, an *E* geometry is indicated for the Δ^7-double bond, and hence the H_3-19 methyl group was placed *trans* to H-7.[13−15] The H_2-9 resonances (δ 2.81, 2.05, m) were seen to be coupled with the H-10 resonance (δ_H 5.02, brs, $W_{1/2}$ = 19.7 Hz), which showed further coupling with the H-11 resonance (δ_H 6.98) of the α,β-unsaturated γ-lactone. The lactone carbonyl carbon exhibited correlations in the HMBC spectrum with the H_2-13 resonances (δ_H 2.15, 2.35, m). The H_2-13 resonances were coupled to the two H-14 resonances (δ_H 1.95 (m), 1.50 (m)), which showed coupling with the previously assigned H-1 resonance. H-1 was arbitrarily assigned as α, as in compound **1a**,[7] and exhibited a correlation in the NOESY spectrum with the H_3-18 methyl group, indicating it was also α and then the methoxy group at C-4 was in the β configuration. A correlation between the methoxy group proton resonance and the isopropyl group resonances (H_3-16 and H_3-17) was also observed, supporting a β configuration of the isopropyl group. The broad multiplet ($W_{1/2}$ = 19.7 Hz) common in the series isolated from the bark and leaves of the same species indicated that H-10 is β in all the compounds isolated.[7] The H-10 resonance showed correlations in the NOESY spectrum with H-11 and the H_3-19 proton resonances, showing that both are on the β-face of the molecule, as illustrated in Figure 1. The conformation adopted by the

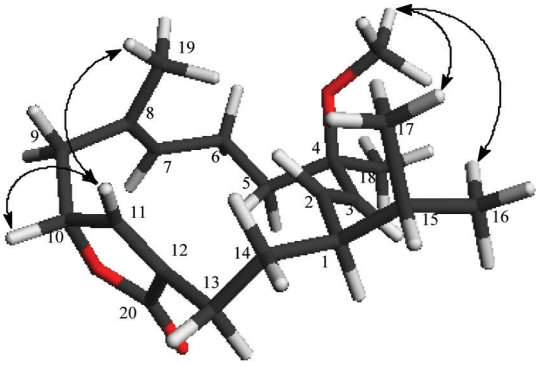


Figure 1. Selected NOE correlations for compound **1**.

molecule results in substituents attached to sp^2 carbons, pointing above or below the general plane of the molecule. The specific rotation for compound **1** was found to be −11.5. Therefore, the structure of compound **1** was determined as (−)-(1*R**,4*R**,10*R**)-4-methoxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide.

The HRMS of compound **2** indicated a molecular formula of $C_{21}H_{32}O_4$, and it was found to be the 1-hydroxy analogue of compound **1**. In addition to a carbonyl stretch at 1754 cm^{-1}, the IR spectrum showed an OH stretching at 3406 cm^{-1}. The ^1H NMR and ^{13}C NMR resonances were similar to those of compound **1** except for a fully substituted oxygenated carbon resonance at δ_C 76.7, instead of the C-1 methine resonance. This was confirmed by HMBC correlations seen between the C-1 resonance and the H-3, H_3-16, H_3-17, and H-15 resonances. Thus a hydroxy group was placed at C-1 in compound **2**. The NOESY spectrum showed a correlation between the H-4β methoxy group proton resonance (δ_H 3.13, s) and the isopropyl group H_3-16 (δ_H 0.91 d J = 7.0 Hz) and H_3-17 (δ_H 0.88 d J = 7.0 Hz) resonances. Thus, these groups were placed on the β face, as in compound **1**, and the hydroxy group was placed in the α orientation. The specific rotation for **2** was found to be −46.4, and this compound was assigned as

dx.doi.org/10.1021/np2002012 | *J. Nat. Prod.* 2011, 74, 2349−2355

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−10 and 8a in $CDCl_3$.

number	1	2	3	4	5	6	7	8	9	10	8a
1	1.62[a]										
2	5.40[a]	5.51 d (16.0)	5.73 d (15.5)	5.57 d (16.1)	6.43 d (11.5)	6.45 d (11.0)	6.19[a]	5.46 d (16.2)	5.47 d (16.1)	5.45 d (16.2)	5.44 d (16.2)
3	5.40[a]	5.74 d (16.0)	5.77 d (15.5)	5.76 d (16.1)	6.06 d (11.5)	6.29 d (11.0)	6.20[a]	5.83 d (16.2)	5.80 d (16.1)	5.71 d (16.2)	5.80 d (16.2)
4											
5α	2.00 m	2.00 m	1.81 m	1.83 m	2.26 m	2.79[a]	3.59 dd (3.6, 7.8)	1.79 m	1.79 m	2.47 dd (3.4, 14.5)	1.76[a]
5β	1.40 m	1.38 m	1.65 m	1.58 m	2.17[a]	2.00 dt (4.0, 3.6)		1.35 m	1.30 m	2.37 m	1.31 m
6α	2.35 m	2.35 m	2.20 m	2.21 m	2.31 m	2.52 m	2.40[a]	1.68[a]	1.70[a]	5.64 m	1.81 s
6β	2.35 m	2.05 m	2.05 m	2.09 m	2.14 m	2.16 d (16.7)	2.30 m	1.36 m	1.50 m		1.48 m
7	5.40[a]	5.30 t (6.0)	5.32 t (6.0)	5.22 t (6.9)	5.08 brs[a]	4.96[a]	5.08 brs	4.16 t (7.1)	3.93 t (7.2)	5.55 d (16.4)	4.89 m
8											
9α	2.05 m	2.30 m	2.46 dd (5.1, 12.9)	2.51[a]	2.17[a]	1.69 s	2.10 m	2.40[a]	2.32 m	1.93 m	2.50 dd (4.8, 14.6)
9β	2.81 dd (5.6, 12.5)	2.53 dd (5.2, 12.8)	2.52 dd (5.1, 12.9)	2.51[a]	2.68[a]	2.79[a]	2.75 dd (5.2, 12.9)	2.80 dd (4.8, 14.9)	2.80 dd (4.9, 14.9)	2.62 dd (5.7, 12.8)	2.77 dd (4.8, 14.6)
10	5.02 brs $W_{1/2}$ = 19.7	5.05 brs $W_{1/2}$ = 16.2	5.08 brs $W_{1/2}$ = 20.7	5.10 m	4.95 brs $W_{1/2}$ = 18.8	4.93[a]	4.98 brs $W_{1/2}$ = 19.9	5.17 brs[a]	5.14 brs $W_{1/2}$ = 19.8	5.13 brs $W_{1/2}$ = 18.0	5.10[a]
11	6.98 d (1.8)	6.83 s	6.91 s	6.87 d (1.5)	7.27 s	7.23 d (1.3)	7.24 d (1.5)	6.88 s	6.90 s	7.16 s	6.93 d (1.6)
12											
13α	2.15 m	2.45 m	2.45 m	2.41 m	2.55 m	2.70 m	2.55 m	2.42[a]	2.40[a]	2.30[a]	2.39[a]
13β	2.35 m	2.35 m	2.35 m	2.36 m	2.45 m	2.34 m	2.45 m	2.40[a]	2.40[a]	2.30[a]	2.39[a]
14α	1.95 m	2.15 m	2.00 m	2.11 m	2.86 m	2.92 td (3.8, 9.1)	2.58[a]	2.05 m	2.01 m	2.05 m	2.05[a]
14β	1.50 m	1.85 m	1.85 m	1.79 m	2.67[a]	2.64 s	2.58[a]	1.80 m	1.82 m	1.78 m	1.78[a]
15	1.62[a]	1.70 sp (7.0)	1.69 m	1.67 sp (6.9)			2.49[a]	1.70[a]	1.70[a]	1.64 sp (6.8)	1.71 sp (6.9)
16	0.91 d (6.6)	0.91 d (7.0)	0.91 d (7.0)	0.90 d (6.9)	5.02 brs	4.96[a]	1.11 d (6.9)	0.90 d (6.8)	0.90 d (6.6)	0.87 d (6.8)	0.92 d (6.8)
					5.08[a]	4.96[a]					
17	0.86 d (6.6)	0.88 d (7.0)	0.86 d (7.0)	0.86 d (6.9)	1.96 s	1.88[a]	1.07 d (6.9)	0.84 d (6.8)	0.87 d (6.6)	0.84 d (6.8)	0.87 d (6.8)
18	1.25 s	1.26 s	1.30 s	1.31 s	1.79 s	1.88[a]	1.67 d (1.2)	1.36 s	1.38 s	1.37 s	1.35 s
19	1.63 s	1.64 s	1.58 s	1.60s	1.58 s	1.67 s	1.59 s	5.29 s	5.19 s	1.39 s	5.28 s
								5.20 s	5.03 s		5.11 s
OCH_3	3.15 s	3.13 s					3.26 s				2.03 s
Ac											
OH										8.60 brs	

[a]Values overlapping.

dx.doi.org/10.1021/np200201t|J. Nat. Prod. 2011, 74, 2349–2355

Table 2. ^{13}C NMR Spectroscopic Data for Compounds 1−10 and 8a in $CDCl_3$.

position	1	2	3	4	5	6	7	8	9	10	8a
1	48.9 CH	76.7 C	76.7 C	77.0 C	142.9 C	143.3 C	147.3 C	77.0 C	77.0 C	76.8 C	77.0 C
2	134.0 CH	130.0 CH	130.6 CH	131.9 CH	124.3 CH	124.2 CH	125.1 CH	131.5 CH	131.3 CH	131.8 CH	131.7 CH
3	135.8 CH	134.2 CH	135.0 CH	135.8 CH	123.6 CH	123.4 CH	118.9 CH	135.9 CH	136.0 CH	135.5 CH	136.2 CH
4	77.2 C	77.2 C	74.3 C	72.9 C	139.9 CH	141.5 C	134.4 C	72.6 C	72.6 C	72.4 C	72.9 C
5	43.7 C	42.0 C	42.1 C	42.4 CH_2	40.3 CH_2	31.4 CH_2	86.0 C	38.6 CH_2	38.8 CH_2	45.2 CH_2	38.5 CH_2
6	25.5 CH_2	21.8 CH_2	23.8 CH_2	23.2 CH_2	25.8 CH_2	26.6 CH_2	31.4 CH_2	25.1 CH_2	29.6 CH_2	127.4 CH	27.4 CH_2
7	131.7 CH	131.7 CH	130.2 CH	131.8 CH	130.0 CH	129.6 CH	123.0 CH	89.8 CH	76.8 CH	134.8 CH	77.9 CH
8	129.4 C	129.0 C	129.9 C	128.1 C	129.9 C	129.8 C	131.6 C	142.0 C	145.3 C	83.6 C	141.2 C
9	44.8 CH_2	43.5 CH_2	42.7 CH_2	42.4 CH_2	44.5 CH_2	46.0 CH_2	44.7 CH_2	34.6 CH_2	34.0 CH_2	41.3 CH_2	36.3 CH_2
10	80.3 CH	80.7 CH	80.9 CH	80.6 CH	79.8 CH	79.1 CH	80.0 CH	79.6 CH	79.8 CH	79.1 CH	79.4 CH
11	149.6 CH	149.4 CH	148.8 CH	148.5 CH	149.3 CH	150.4 CH	149.3 CH	146.5 CH	147.0 CH	150.2 CH	145.8 CH
12	133.5 C	134.2 C	135.0 C	135.3 C	133.9 C	131.6 C	133.7 C	135.9 C	135.3 C	133.1 C	136.3 C
13	22.0 CH_2	22.0 CH_2	20.9 CH_2	21.3 CH_2	23.7 CH_2	23.7 CH_2	22.5 CH_2	20.7 CH_2	20.8 CH_2	20.2 CH_2	20.4 CH_2
14	28.5 CH_2	33.7 CH_2	35.3 CH_2	33.7 CH_2	24.7 CH_2	23.3 CH_2	27.3 CH_2	34.6 CH_2	34.8 CH_2	35.0 CH_2	34.5 CH_2
15	34.0 CH	39.4 CH	37.5 CH	38.9 CH	137.9 C	137.3 C	32.4 CH	38.8 CH	38.5 CH	38.9 CH	38.4 CH
16	20.7 CH_3	17.8 CH_3	17.7 CH_3	17.1 CH_3	113.0 CH_2	113.4 CH_2	23.0 CH_3	17.7 CH_3	17.7 CH_3	17.8 CH_3	17.8 CH_3
17	19.5 CH_3	16.9 CH_3	16.6 CH_3	17.5 CH_3	21.7 CH_3	21.6 CH_3	23.0 CH_3	16.8 CH_3	16.8 CH_3	16.8 CH_3	16.8 CH_3
18	22.3 CH_3	23.0 CH_3	31.1 CH_3	28.8 CH_3	16.5 CH_3	24.0 CH_3	12.5 CH_3	29.9 CH_3	29.7 CH_3	29.5 CH_3	30.6 CH_3
19	16.3 CH_3	17.1 CH_3	17.5 CH_3	17.9 CH_3	16.8 CH_3	16.1 CH_3	16.5 CH_3	118.4 CH_2	115.7 CH_2	25.8 CH_2	119.1 CH_2
20	174.2 C	174.4 C	174.5 C	174.5 C	174.5 C	174.6 C	174.4 C	174.0 C	174.1 C	175.0 C	173.8 C
OCH_3	50.4 CH_3	50.4 CH_3					56.5 CH_3				
OAc											21.4
OAc											171.1

dx.doi.org/10.1021/np200202t|J. Nat. Prod. 2011, 74, 2349–2355

(−)-(1*S**,4*R**,10*R**)-1-hydroxy-4-methoxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide.

Compound **3**, $C_{20}H_{30}O_4$, was assigned as (−)-(1*S**,4*S**,10*R**)-1,4-dihydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide. The IR spectrum gave an absorption band at 3421 cm^{-1} for a hydroxy group and an α,β-unsaturated γ-lactone absorption band at 1757 cm^{-1}, as was observed for **1** and **2**. Compound **3** was found to be a 4-hydroxy analogue of **2**. Its NMR spectra were very similar to those of **2**, but the methoxy group proton resonance present in **1** and **2** was absent and the C-4 and C-18 resonances of **3** were shifted to δ_C 74.3 (δ_C 77.2 for **2**) and δ_C 31.1 (δ_C 23.0 for **2**), respectively. The relative configuration for **3** was assumed to be the same as that of **2**.

Compound **4**, $C_{20}H_{30}O_4$, was found to be the C-4 epimer of **3**. This was confirmed by the correlation between the H_3-18 (1.31, s) resonance and the isopropyl group H_3-16 (δ_H 0.90, d, J = 6.9 Hz) and H_3-17 (δ_H 0.86, d, J = 6.9 Hz) resonances in the NOESY spectrum. The ^{13}C NMR chemical shift for the oxygenated C-4 (δ_C 72.9) and C-18 (δ_C 28.8) for **4** differed slightly from those of **3** (δ_C 74.3 and 31.1). The structure of compound **4** was proposed as (−)-(1*S**,4*S**,10*R**)-1,4-dihydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide. Compound **4** is the 1α-hydroxy derivative of compound **4a**, previously isolated from the bark and reisolated from the leaves in the present investigation.

Compound **5**, $C_{20}H_{26}O_2$, was identified as (+)-(10*R**)-cembra-1*E*,3*E*,7*E*,11*Z*,15-pentaen-20,10-olide. The IR spectrum showed no OH band, and the NMR spectra differed in having an additional three trisubstituted double bonds and a terminal methylene group. The observation of coupled terminal methylene group proton resonances at δ_H 5.02 (s) and 5.08 (s) and a vinyl methyl three-proton resonance at δ_H 1.96, instead of the H_3-16 and H_3-17 doublets present in the ^1H NMR spectra of compounds **1**−**4**, indicated the presence of an isopropenyl group at C-1 in **5**. The C-1 resonance (δ_C 142.9) showed correlations in the HMBC spectrum with the two H_2-16 and H_3-17 proton resonances and resonances at δ_H 6.43 (H-2, J = 11.5 Hz) and 6.06 (H-3, J = 11.5 Hz). The corresponding C-3 resonance (δ_C 123.6) correlated in the HMBC spectrum with the H_3-18 vinyl methyl group proton resonance at δ_H 1.79, indicating a conjugated Δ^1,Δ^3-system. The NOESY spectrum showed a correlation between the terminal methylene proton resonances and the H-2 resonance and between the H-2 resonance and the H_3-18 methyl group proton resonance. Thus it was deduced that both double bonds were *E*. The chemical shift of δ_C 16.5 for C-18 supported this deduction.[15] These coupling constants and chemical shifts were in agreement with those found for crotocembraneic acid and neocrotocembraneic acid, which also have a Δ^1,Δ^3-double bond system.[16,17] A specific rotation value of +24.2 for **5** was determined.

Compound **6**, $C_{20}H_{26}O_2$, was found to be a geometric isomer of **5**. The most significant difference between the ^{13}C NMR spectra of these two compounds was the differences in chemical shifts for the C-5 and C-18 carbon resonances, which occurred at δ_C 40.3 and 16.5, respectively, in **5** but at δ_C 31.4 and 24.0 in **6**. The value of the chemical shift of δ_C 24.0 for the C-18 vinylic methyl group carbon resonance was indicative of a *Z* configuration of the Δ^3-double bond,[15] and this was confirmed by correlations in the NOESY spectrum between H-3 and the H_3-18 resonance. A specific rotation value of +18.6 was measured for **6**. This compound was assigned as (+)-(10*R**)-cembra-1*Z*,3*Z*,7*E*,11*Z*,15-pentaen-20,10-olide.

Compound **7**, $C_{21}H_{30}O_3$, as in compound **5**, has a Δ^1,Δ^3-conjugated system with an isopropyl substituent at C-1 and a methoxy group at C-5. The methoxy group three-proton singlet resonance (δ_H 3.26) showed a correlation in the HMBC spectrum with an oxymethine resonance (δ_C 86.0), which showed a further correlation with the H_3-18 proton resonance (δ_C 1.76). The corresponding C-18 resonance was shifted markedly upfield to δ_C 12.5. The NOESY spectrum exhibited correlations between the H-10β resonance and the H_3-19 resonance, which, in turn, correlated with one of the H-6 (δ_H 2.40) and H-5 (δ_H 3.59 dd J = 3.60, 7.80 Hz) resonances. Thus, these protons were assigned as β. A specific rotation value of +24.2 was measured for **7**. This compound was assigned as (+)-(5*R**,10*R**)-5-methoxycembra-1*E*,3*E*,7*E*,11*Z*, 15-pentaen-20,10-olide.

Compounds **8** and **9** were found to be C-7 epimers of each other, and HRESIMS analysis indicated both had a molecular formula of $C_{20}H_{30}O_3$. As with compound **4**, they both had an isopropyl group at C-1 and hydroxy groups at C-1α and C-4α, but the Δ^7-double bond had moved to the 8,19-position and a hydroxy group was found to be present at C-7. For compound **8** this was confirmed by correlations seen in the HMBC spectrum between the C-8 resonance (δ_C 142.0) and one of the two methylene protons at C-19 resonances (δ_H 5.29) and between the C-19 resonance (δ_C 29.9) and H-7 (δ_H 4.16, t, J = 7.1 Hz) and one of the H-9 resonances (δ_H 2.40). In the COSY spectrum the H-7 resonance coupled to the two H-6 proton resonances, which were further coupled to the two H-5 resonances in the COSY spectrum. Similar correlations were observed in the NMR spectra of **9**. The ^{13}C NMR chemical shift values for C-6, C-7, and C-19 differed considerably between compounds **8** and **9** (see Table 2). As with compound **4**, the relative configuration of **8** and **9** could be determined using the NOESY spectrum. In both cases, correlations could be seen between the isopropyl group methyl and H_3-18 proton resonances, and so these groups were assigned as β. For **8**, correlations were also observed in the NOESY spectrum between the H_3-18 methyl group and the proton resonance and the H-7 proton resonance, which showed, in turn, a correlation with the H_2-19 proton resonance. The latter correlated with the H-11 proton resonance, which, in turn, showed a correlation with the H-10 resonance, establishing these as occurring on the β face, and therefore the hydroxy groups at C-1, C-4, and C-7 as α. The specific rotation for compound **8** was +100. Compound **8** was therefore assigned as (+)-(1*S**,4*S**,7*R**,10*R**)-1,4,7-trihydroxycembra-2*E*,8-(19),11*Z*-trien-20,10-olide. The change in the ^{13}C NMR chemical shifts for the oxymethine C-7 from δ_C 89.8 in compound **8** and δ_C 76.8 for compound **9** indicated a different configuration at C-7. This was confirmed by a lack of any correlation between H-7 and H_3-18 in the NOESY spectrum of **9**. Thus the C-7 hydroxy group was assigned as β in **9**. The specific rotation of **9** was −24.8, and it was assigned as (−)-(1*S**,4*S**,7*S**,10*R**)-1,4,7-trihydroxycembra-2*E*,8-(19),11*Z*-trien-20,10-olide.

Compound **10**, $C_{20}H_{30}O_5$, displayed in its NMR spectra, in addition to resonances ascribable to a α,β-unsaturated γ-lactone, three oxygenated fully substituted carbon resonances and two alkene double bonds. The resonance at δ_C 76.8 showed a correlation with the two isopropyl group methyl doublets in the HMBC spectrum and was assigned to C-1. This resonance also showed correlations with two olefinic proton resonances at δ_H 5.45, d, J = 16.2 Hz, and 5.71, d, J = 16.2 Hz,

dx.doi.org/10.1021/np2002012 | J. Nat. Prod. 2011, 74, 2349−2355

which could be assigned as H-2 and H-3 of a *trans*-disubstituted double bond. The C-3 resonance showed correlations with the C-4 oxygenated carbon (δ_C 72.4), the C-18 methyl group carbon resonances, and the CH_2-5 carbon resonance. The corresponding H-5 resonances showed coupling with the H-6 olefinic resonance [δ_H 5.64 (m)], which was further coupled to the H-7 resonance (δ_H 5.55, d, J = 16.4 Hz) of the second *trans*-disubstituted double bond. The H-7 resonance showed correlations in the HMBC spectrum with the oxygenated C-8 resonance (δ 83.6) and the C-19 methyl group proton resonance (δ_C 25.8).

The relative configuration of **10** was assigned using the NOESY spectrum. The two methyl proton resonances of the isopropyl group H_3-16 (δ_C 0.84, d, J = 6.8 Hz) and H_3-17 (δ 0.87, d, J = 6.8 Hz) showed correlation with the H_3-18 (δ 1.37, s) methyl proton resonance, as was seen for compounds **4**, **8**, and **9**, and were likewise placed on the β face, as was H-10. No correlations were seen in the NOESY spectrum with the H_3-19 resonance, and so this group was assigned as α with the C-8 hydroxy group β. The specific rotation of +32.0 was determined, and **10** was assigned as (+)-(1*S**,4*R**,8*S**,10*R**)-1,4,8-trihydroxycembra-2*E*,6*E*,11*Z*-trien-20,10-olide.

The acetate derivative of **8** was prepared in an attempt to produce a crystalline product for single-crystal X-ray analysis or a more stable compound for screening. Unfortunately, the derivative was not crystalline but was stable enough to undergo in vitro antiplasmodial screening against *P. falciparum* (CQS) D10 strain. Compounds **4a** and **8a** exhibited IC_{50} values of 20.8 and 13.5 μg/mL, respectively, compared to chloroquine (IC_{50} 27.0 ng/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were measured at room temperature in chloroform using a JASCO P-1020 polarimeter, and IR spectra were recorded using a Perkin-Elmer (2000 FTIR) spectrometer. Samples were dissolved in chloroform and analyzed using NaCl plates. NMR spectra were recorded on a Bruker AVANCE 500 NMR spectrometer in deuteriochloroform ($CDCl_3$), and the chemical shifts were recorded in ppm (parts per million) relative to the deuteriochloroform central line at δ 7.260 in the ^{1}H NMR spectrum and at δ 77.23 in the ^{13}C NMR spectrum. HRESIMS were determined using a Bruker MicroToF mass spectrometer, with an Agilent 1100 HPLC used to introduce the samples.

Plant Material. Leaves of *Croton gratissimus* Burch. var. *gratissimus* were collected in August 2005 from a mature tree cultivated on the campus of the University of KwaZulu-Natal, Durban, South Africa, by one of the authors (N.R.C.). A voucher (*Crouch 1051*) is retained for verification purposes at the KwaZulu-Natal Herbarium (NH).

Extraction and Isolation. The ground leaves (189 g) of *C. gratissimus* were extracted using a Soxhlet apparatus for 24 h successively using hexane, methylene chloride, ethyl acetate, and methanol. After evaporation of the solvent in vacuo the following extracts were obtained: hexane (8.46 g), methylene chloride (2.55 g), ethyl acetate (8.84 g), and methanol (25.57 g). The hexane and methylene chloride extracts were combined due to their similar TLC bands. The combined hexane and methylene chloride extracts as well as the ethyl acetate extracts were examined in this study. Column chromatography of combined hexane and methylene chloride extracts and ethyl acetate extract over silica gel (Merck 9385) using a 5 cm diameter by 60 cm length gravity column, collecting fractions (75 mL each) using a hexane/methylene chloride step gradient starting with 100% hexane and gradually increasing the methylene chloride concentration to 100%, followed by 5% methanol in methylene chloride, was used to separate the constituents. Final purification was undertaken over silica gel using a 1 cm diameter by 60 cm length gravity column with different solvents as indicated below for each compound. The combined hexane and methylene chloride extracts gave the following compounds: fractions 40−54 were repurified using CH_2Cl_2/hexane, 1:1, to give **5** (30 mg) and **6** (19 mg); fractions 60−73 gave α-glutinol (47 mg) using 100% CH_2Cl_2, and fractions 100−127 gave **4** (30 mg) using 100% CH_2Cl_2. The ethyl acetate extract gave fractions 10−19 (20% CH_2Cl_2 in hexane), which gave (+)-(1*R**,10*R**)-cembra-2*E*,4*E*,7*E*,11*Z*-tetraen-20,10-olide (13 mg); fractions 21−26 (100% CH_2Cl_2) gave 24-ethylcholesta-4,22-dien-3-one (31.5 mg); fractions 27−40 (100% CH_2Cl_2) gave lupeol (26 mg) and **2** (17.0 mg); fractions 45−67 (100% CH_2Cl_2) gave additional **3** (10 mg) and eudesm-4(15)-ene-1β,6α-diol (15 mg); fractions 78−123 (3% MeOH/97% CH_2Cl_2) gave (+)-(1*R**,4*S**,10*R**)-4-hydroxycembra-2*E*,7*E*,11*Z*-trien-20,10-olide (**4a**) (13 mg), **8** (9.7 mg), **9** (6.5 mg), and **10** (30 mg). The ^{1}H and ^{13}C NMR spectroscopic data for all new compounds are shown in Tables 1 and 2, respectively.

(−)-(1R,4R*,10R*)-4-Methoxycembra-2E,7E,11Z-trien-20,10-olide (**1**):* colorless oil; $[\alpha]^{24}_D$ −11.5 (*c* 0.0013, $CHCl_3$); IR (neat) ν_{max} 2938, 2853, 1759 cm^{-1}; HRESIMS *m*/*z* 355.1882 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_3Na$, 332.1984).

(−)-(1S,4R*,10R*)-1-Hydroxy-4-methoxycembra-2E,7E,11Z-trien-20,10-olide (**2**):* colorless oil; $[\alpha]^{24}_D$ −46.4 (*c* 0.0076, $CHCl_3$); IR (neat) ν_{max} 3406, 2929, 2861, 1754 cm^{-1}; HRESIMS *m*/*z* 371.2188 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_4Na$, 348.2300).

(−)-(1S,4S*,10R*)-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide (**3**):* colorless oil; $[\alpha]^{24}_D$ −12.9 (*c* 0.004, $CHCl_3$); IR (neat) ν_{max} 3421, 2938, 2851, 1757; HRESIMS *m*/*z* 357.2027 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_4Na$, 321.2144).

(−)-(1S,4S*,10R*)-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide (**4**):* colorless oil; $[\alpha]^{24}_D$ −42.0 (*c* 0.0075, $CHCl_3$); IR (neat) ν_{max} 3429, 1740 cm^{-1}; HRESIMS *m*/*z* 357.2037 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

(+)-(10R)-Cembra-1E,3E,7E,11Z,16-pentaen-20,10-olide (**5**):* colorless oil; $[\alpha]^{24}_D$ +24.2 (*c* 0.0004, $CHCl_3$); IR (neat) ν_{max} 2959, 2863, 1753 cm^{-1}; HRESIMS *m*/*z* 321.1825 [M + Na]$^+$ (calcd for $C_{20}H_{26}ONa$, 321.1831).

(+)-(10R)-Cembra-1Z,3Z,7E,11Z,15-pentaen-20,10-olide (**6**):* colorless oil; $[\alpha]^{24}_D$ +18.6 (*c* 0.007, $CHCl_3$); IR (neat) ν_{max} 3063, 2923, 2856, 1749 cm^{-1}; HRESIMS *m*/*z* 321.1819 [M + Na]$^+$ (calcd for $C_{20}H_{26}ONa$, 321.1831).

(+)-(5R,10R*)-5-Methoxycembra-1E,3E,7E,11Z,15-pentaen-20,10-olide (**7**):* colorless oil; $[\alpha]^{24}_D$ +27.6 (*c* 0.0039, $CHCl_3$); IR (neat) ν_{max} 2934, 2828, 1759 cm^{-1}; HRESIMS *m*/*z* 339.1923 [M + Na]$^+$ (calcd for $C_{21}H_{30}O_3Na$, 330.2195).

(+)-(1S,4S*,7R*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide (**8**):* colorless oil; $[\alpha]^{24}_D$ +100 (*c* 0.0004, $CHCl_3$); IR (neat) ν_{max} 3411, 1733, 1645, 847 cm^{-1}; HRESIMS *m*/*z* 373.1978 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

Acetylation of (+)-(1S,4S*,7R*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide (**8**).* Compound **8** (5 mg) was dissolved in pyridine (1 mL) and acetic anhydride (1 mL) and left to stand for 12 h. MeOH (2 × 5 mL) was added, and solvent was removed under vacuum to yield (+)-(1*S**,4*S**,7*R**,10*R**)-7-acetoxy-1,4-dihydroxycembra-2*E*,8(19),11*Z*-trien-20,10-olide (**8a**). The ^{1}H and ^{13}C NMR spectroscopic data of **8a** are shown in Tables 1 and 2, respectively.

(−)-(1S,4S*,7S*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide (**9**):* colorless oil; $[\alpha]^{24}_D$ −24.8 (*c* 0.004, $CHCl_3$); IR (neat) ν_{max} 3428, 1739 cm^{-1}; HRESIMS *m*/*z* 373.1980 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_3Na$, 373.1991).

(+)-(1S,4R*,8S*,10R*)-1,4,8-Trihydroxycembra-2E,6E,11Z-trien-20,10-olide (**10**):* colorless oil; $[\alpha]^{24}_D$ +32.0 (*c* 0.001, $CHCl_3$); IR (neat) ν_{max} 3390, 1730 cm^{-1}; HRESIMS *m*/*z* 373.1964 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

In Vitro Antiplasmodial Activity of Compounds 4a and 8a against *P. falciparum* (CQS) D10 Strain. The test samples were tested in duplicate against the chloroquine-sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[18] Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[19]

The two extracts were dissolved and diluted to give a 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment in complete medium. Chloroquine was used as the reference drug in all experiments. A full dose−response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 100 μg/mL, which was serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 0.2 μg/mL. Chloroquine was tested at a starting concentration of 100 ng/mL. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a nonlinear dose−response curve fitting analysis using Graph Pad Prism v. 4.0 software.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**10** are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

This research was funded by the University of Surrey through a Ph.D. studentship for M.L. and the South African National Research Foundation (NRF).

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