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New clerodane diterpenoids from *Laetia procera* (Poepp.) Eichler (Flacourtiaceae), with antiplasmodial and antileishmanial activities

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Abstract—Extracts of *Laetia procera* (Flacourtiaceae) displayed significant in vitro activity against *Plasmodium falciparum*. *P. falciparum* bioassay guided fractionation of a trunk bark extract of this plant led to the isolation of six clerodane diterpenoids (**1**–**6**) and a butanolide (**7**). Five of these compounds are new and called Laetiaprocerine A–D (**3**–**6**) and Laetianolide A (**7**). Their structures were established on the basis of 1D and 2D NMR experiments. Absolute configurations of **1** and **2** were determined by a modified Mosher's method and the absolute configuration of **5** by chemical correlation. The clerodane diterpenoids displayed activities against *P. falciparum* with an IC_{50} down to 0.5 μM on FCb1 and F32 strains, and also cytotoxicity toward human tumor cell line MCF7. The most active compound showed a selectivity index of 6.8. Some of these compounds also displayed activities against *Leishmania amazonensis* amastigote axenic stages and promastigote.

During our search for new bioactive agents from the biodiversity of French Guiana, extracts of *Laetia procera* (Poepp.) Eichler (Flacourtiaceae) showed significant activity on *Plasmodium falciparum* screening in vitro. *P. falciparum* bioguided fractionation allowed six clerodane diterpenes (compounds **1**–**6**) and a new butanolide **7** (Fig. 1) to be isolated. Four of the clerodane diterpenoids are new (**3**–**6**). Such terpenoids have been found previously in the leaves of *Laetia procera* and in the fruits of *Laetia corymbosa*.[1,2] The genus *Casearia* (Flacourtiaceae) has also been widely studied for the isolation of clerodane diterpenoids.[3,4] Similar compounds have been reported in *Bucida bucera* (Combretaceae) and *Licania intrapetiolaris* (Chrysobalanaceae).[5,6] The biological activities displayed by these products are mostly in vitro cytotoxicity on various tumor cell lines.[7–10] Some of them are active on Sarcoma 180 ascites in mice.[11] They also show immunomodulato-

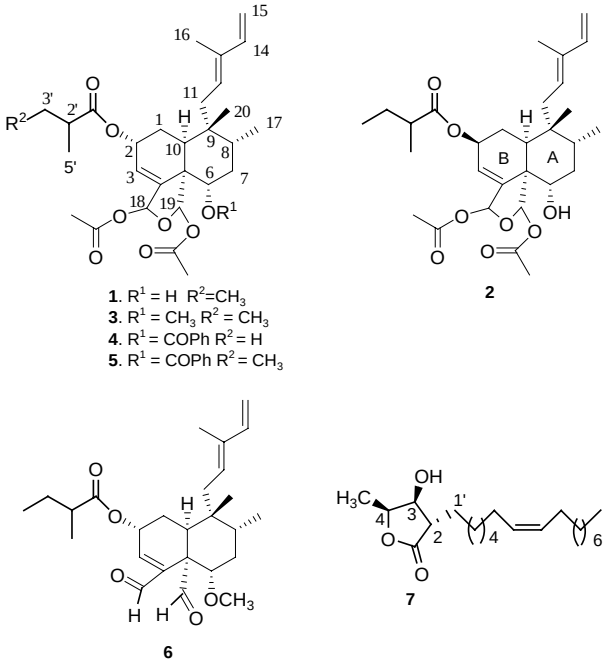

Figure 1. Compounds isolated from *Laetia procera*.

Keywords: *Laetia procera*; Flacourtiaceae; Clerodane diterpenoids; Butanolide; *Plasmodium falciparum*; Leishmania.
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ry properties and trypanocidal activity.[12,13] Recently, antiplasmodial activity and a moderate activity against *Mycobacterium tuberculosis* have been reported.[3] We believe that the present work is the first report of the antileishmanial activity of such compounds.

The trunk barks were collected in French Guiana, in the Saint Elie tropical rain forest. This sampling spot is a permanent investigation area containing up to 800 identified trees. The systematic identification of the trees was performed at the IRD herbarium in Cayenne where a voucher sample is deposited (Accessing No. Prévost 1120).[14]

Powdered trunk bark of *L. procera* was subjected to successive extractions with solvents of increasing polarity (cyclohexane, dichloromethane, ethyl acetate, and methanol). The highest antiplasmodial activity was found for the cyclohexane fraction. This extract was fractionated by flash chromatography on silica gel using dichloromethane containing increasing amounts of methanol (0% to 10%). The antiplasmodial activity was concentrated in the fraction eluted with 1% to 3% of methanol. Further fractionation by successive flash chromatography on silica gel led to the isolation of the pure compounds Casearlucine A **1** and Caseamembrol A **2**. NMR and mass spectral data of compounds **1** and **2** were identical to those previously reported for Casearlucine A (or Bucidarasin B) and Caseamembrol A,[9,5,10] although compound **2** displayed a significantly higher optical rotation than the value reported for Caseamembrol A (reported for Casearmembrol A: $[\alpha]_D$ −8.3° (*c* 0.38, MeOH), found for **2**: $[\alpha]_D$ −61° (*c* 0.40, MeOH)). Laetiaprocerines A–D and Laetianolide A were obtained by further purification on reversed-phase semi-preparative HPLC.[15]

The structures of Laetiaprocerines A-D were determined by careful analysis of their NMR data, which are summarized in Tables 1 and 2. Laetiaprocerine C **5** ($C_{36}H_{46}O_9$, HRTOFESIMS) displayed ^1H and ^{13}C spectra similar to those of the known Casearlucine A **1**, but showed additional signals assigned to a benzoic ester moiety. NMR data of **5** could be fully assigned as follows: the COSY spectrum allowed us to assign the two H_7 (1.75 and 1.98 ppm) through their correlation with H_6 (5.21 ppm). The signal at 1.98 ppm also showed a strong COSY correlation with a methyl doublet at 0.97 ppm. The H_7 signal at 1.75 ppm only showed a COSY correlation with its vicinal proton at 1.98 ppm. The proton signal of H_8 overlapped with H_7 at 1.98 ppm: the HSQC spectra showed that the signal at 1.98 ppm correlated with C_7 at 33.0 ppm and also with a carbon at 36.2 ppm, which could be assigned to C_8. This was confirmed by the HMBC correlations of the signal at 36.2 ppm with the methyl doublet at

Table 1. ^1H NMR data of **1**–**6**[a]

Protons	1	2	3	4	5	6
1a	1.92 m	1.70 m	1.93 m	1.99 m	2.01 m	1.83 m
1b	1.92 m	2.19 m	1.93 m	2.12 m	2.1 m	1.94 br d 15.2
2	5.46 m	5.63 m	5.46 m	5.48 m	5.48 m	5.53 m
3	6.02 dd 4.3–1.6	5.91 br s	5.96 dd 4.3–1.6	6.01 br d 4.3	6.02 br d 4.3	6.87 m
6	3.82 m	4.03 dd 12.0–3.7	3.33 dd 12.4–2.8	5.21 dd 12.1–4.0	5.21 dd 12.7–3.8	3.39 m
7a	1.63 m	1.65 m	1.46 q 12.8	1.75 m	1.75 m	1.77 m
7b	1.77 m	1.81 m	1.90 d 14.2	1.99 m	1.98 m	2.05 m
8	1.79 m	1.88 m	1.72 m	1.98 m	1.97 m	1.73 m
10	2.39 t 8.5	2.41 m	2.40 dd 9.5–7.5	2.51 dd 13.5–3.2	2.52 dd 14.3–7.2	2.49 dd 10.2–3.2
11a	1.70 m	1.68 m	1.70 d 16.4	1.81 d 17.2	1.81 d 17.8	1.76 m
11b	2.25 dd 16.9–8.3	2.25 dd 17.4–8.5	2.25 dd 16.8–8.6	2.31 dd 17.2–8.0	2.31 dd 17.5–7.8	2.27 dd 15.8–9.0
12	5.39 m	5.39 m	5.40 m	5.44 m	5.43 m	5.31 m
14	6.28 dd 17.3–10.7	6.33 dd 17.4–10.6	6.28 dd 17.4–10.7	6.33 dd 17.3–10.7	6.33 dd 17.3–10.7	6.39 dd 17.4–10.6
15a	4.95 d 10.7	4.97 d 10.6	4.94 d 10.6	4.98 d 10.7	4.98 d 10.7	4.95 d 10.6
15b	5.12 d 17.3	5.12 d 17.4	5.11 d 17.3	5.14 d 17.3	5.14 d 17.4	5.09 d 17.5
16	1.63 s	1.68 s	1.68 s	1.71 s	1.71 s	1.73 s
17	0.95 d 6.7	0.96 d 7.5	0.97 d 6.4	0.98 d 6.7	0.97 d 6.6	1.02 d 6.9
18	6.75 br s	6.72 br s	6.67 br s	6.56 br s	6.56 br s	9.38 s
19	6.53 s	6.49 s	6.5 s	6.81 s	6.81 s	10.42 s
20	0.83 s	0.87 s	0.82 s	0.87 s	0.89 s	0.85 s
2′	2.49 m	2.41 m	2.49 m	2.67 sept 7.0	2.50 m	2.46 m
3′a	1.59 m	1.53 m	1.57 m	1.24 d 6.9	1.59 m	1.53 m
3′b	1.72 m	1.72 m	1.72 m		1.73 m	1.70 m
4′	0.99 t 7.3	0.95 t 7.5	0.99 t 7.4		1.00 t 7.4	0.94 t 7.4
5′	1.20 d 6.9	1.18 d 7.0	1.20 d 6.96	1.25 d 6.9	1.21 d 7.0	1.17 d 6.9
Me-18	2.10 s	2.12 s	2.11 s	2.08 s	2.07 s	
Me-19	1.95 s	1.97 s	1.95 s	2.06 s	2.00 s	
2″						
3″				8.15 br d 8.2	8.15 br d 8.2	
4″				7.48 m	7.48 m	
5″				7.59 m	7.59 m	
OCH_3			3.32 s			3.31 s

[a] All spectra were recorded in $CDCl_3$, 500 MHz.

Table 2. ^{13}C NMR data of **1**–**6**[a]

Carbon[b]	1	2	3	4	5	6
1	26.7	26.1	26.9	26.7	26.6	25.8
2	66.2	70.4	66.1	65.8	65.9	64.7
3	121.8	124.3	121.2	123.2	123.2	140.2
4	145.3	144.2	146.1	144.1	144.3	148.7
5	53.5	53.5	52.9	51.9	51.9	55.1
6	72.8	74.1	81.7	74.5	74.5	81.5
7	37.3	37.7	31.3	33.0	33.0	31.9
8	36.8	36.8	36.2	36.1	36.2	35.6
9	37.6	38.4	37.7	37.5	37.6	39.0
10	36.8	41.4	36.7	37.3	37.3	40.0
11	30.3	30.0	30.2	30.3	30.3	31.3
12	129.0	128.7	129.3	128.9	128.9	126.7
13	135.7	135.9	135.5	135.8	135.9	136.6
14	141.2	141.1	141.3	141.2	141.2	141.6
15	111.0	111.1	110.9	111.2	111.2	110.7
16	11.9	12.0	11.9	12.0	12.0	12.3
17	15.5	15.6	15.7	15.4	15.4	15.7
18	95.6	95.1	96.2	95.3	95.3	191.2
19	97.0	96.6	97.5	97.5	97.5	202.2
20	24.9	25.0	25.1	25.1	25.0	25.2
1′	175.9	176.5	176.0	176.3	175.8	176.0
2′	41.1	41.1	41.1	34.0	41.1	40.9
3′	26.9	26.8	27.1	18.7	27.0	27.0
4′	11.6	11.7	11.6		11.7	11.6
5′	16.6	16.5	16.6	19.1	16.6	16.3
OCO_{18}	170.1	170.2	170.3	170.0	170.0	
$MeCO_{18}$	21.2	21.2	21.3	21.1	21.1	
OCO_{19}	169.4	169.5	169.5	169.6	169.5	
$MeCO_{19}$	21.5	21.6	21.7	21.7	21.7	
1″				165.7	165.7	
2″				129.4	129.5	
3″				129.9	129.9	
4″				133.5	133.5	
5″				133.5	133.5	
OCH_3			57.5			57.2

[a] All spectra were recorded in $CDCl_3$, 125 MHz.

[b] The J_{mod} experiment allowed us to distinguish between CH_2/C and CH_3/CH.

0.97 ppm and a methyl singlet at 0.89 ppm. So we assigned H_8 and C_8 (1.98–36.2 ppm), and H_{17} and C_{17} (0.97–15.4 ppm). The methyl singlet at 0.89 ppm (carbon at 25.0 ppm) showed HMBC correlations with C_7, C_8 and signals at 37.3, and/or 37.6 ppm: it could therefore be assigned as H_{20}, the signal at 37.6 ppm being C_9. The conjugated double bond system could be unambiguously identified with COSY correlations (between H_{15a}, H_{15b}, and H_{14}), HMBC correlations (C_{14}/H_{16}–C_{14}/H_{12}–C_{13}/H_{15}–C_{13}/H_{16}–C_{13}/H_{11}), and NOESY correlations (H_{15b}/H_{16}–H_{15a}/H_{14}–H_{14}/H_{12}–H_{11}/H_{16}). Proton H_{11} (2.31 ppm) showed HMBC correlations with 25.0, 36.2 ppm (C_8), 37.3 and/or 37.6 ppm, which confirmed the assignment of H_{20} and C_{20} (0.89–25.0 ppm), and C_9 (37.6 ppm). The carbon at 37.3 ppm bore a proton at 2.52 ppm, which showed HMBC correlations with C_{11}, C_8, C_9, and C_6. Signals at 2.52–37.3 ppm could be therefore assigned as H_{10}–C_{10}. Other HMBC correlations of H_{10} with carbons at 26.6, 51.9, 65.9, and 97.5 ppm allowed us to assign these signals to C_1–C_5–C_2–C_{19}, respectively. This assignment was confirmed by the COSY (H_{10}/H_{1a}–H_{10}/H_{1b}– H_2/H_{1a}–H_2/H_{1b}) and the HMBC (C_5/H_6–C_5/H_{19}) correlations. An ethylenic proton at 6.02 ppm displayed HMBC correlations with C_1 and C_5. Proton H_2 also showed COSY correlation with this ethylenic proton allowing us to assign H_3 and C_3 (6.02–123.2 ppm). HMBC correlations of H_3 with signal at 95.3 ppm and of H_2 with signals at 144.3 and 175.8 ppm allowed us to assign H_{18} and C_{18} (6.56–95.3 ppm), C_4 (144.3 ppm), and the carbonyl $C_{1'}$ (175.8 ppm). The secondary butyl side chain was assigned thanks to the HMBC ($C_2/H_{2'}$–$C_2/H_{3'a}$–$C_2/H_{3'b}$–$C_2/H_{5'}$) and the COSY data ($H_{2'}/H_{5'}$–$H_{2'}/H_{3'a}$–$H_{2'}/H_{3'b}$–$H_{4'}/H_{3'a}$–$H_{4'}/H_{3'b}$). HMBC correlation of H_6 with 165.7 ppm showed that C_6 bears an ester ($C_{1''}$ at 165.7 ppm). HMBC correlations of the aromatic signals at 7.48 ppm and 8.16 ppm with this carbonyl indicated that it belongs to a benzoic ester. HMBC and COSY spectra also allowed the assignment of these aromatic signals. H_{19} showed HMBC correlation with the carbon at 169.5 ppm (OCO_{19}) and the methyl carbon at 21.7 ppm (Me-19). H_{18} showed HMBC correlation with the carbon at 170.0 ppm (OCO_{18}) and the methyl at 21.1 ppm (Me-18). All these data allowed us to assign the structure **5** to this new compound, Laetiaprocerine C.

Laetiaprocerine A **3** ($C_{30}H_{44}O_8$, HRTOFESIMS) differed from Casearlucine A **1** by an additional methyl (3.32 ppm; 57.5 ppm). These data indicated that **3** bears

a methoxy group. The chemical shifts of C_6 and H_6 in compound **3** indicated that this methoxy group is on C_6. This hypothesis was fully supported by the 2D NMR spectral analysis of compound **3**. Laetiaprocerine D **6** ($C_{26}H_{38}O_5$, HRTOFESIMS) and Laetiaprocerine A **3** displayed similar NMR data although signals of acetate and methyldioxy groups disappeared and were replaced by two aldehyde groups on C_{18} and C_{19}. Other 2D NMR were in agreement with the structure **6** proposed for this new compound. Laetiaprocerine B **4** ($C_{35}H_{44}O_9$, HRTOFESIMS) was close to compound **5**: it showed an additional methyl signal (1.24 ppm, d, $J = 6.9$ Hz), but also absence of the methyl triplet (1.0 ppm) and of the methylene multiplets (1.59 and 1.73 ppm) observed in **5**. These data suggest that **4** bears an isobutanoyloxy moiety instead of a 2-methylbutanoyloxy group. The location of this group at C_2 was indicated by the HMBC correlations ($H_2/C_{1'}$–$H_{2'}/C_{1'}$–$H_{3'}/C_1$).

The relative stereochemistry of the new clerodane diterpenoids **3**–**6** was determined by a NOESY experiment. The configuration of the double bond between C_{12} and C_{13} was the same for all clerodane diterpenoids described in this paper: a strong NOE effect between H_{12} and H_{14} indicated an *E* configuration. Laetiaprocerine A **3** (Fig. 2): NOE effects between H_{10} and H_{20}, and H_{10} and H_{12} indicated that H_{10} is equatorial for the A ring. Therefore, the junction between C_{10} and C_1 is axial, and a NOE effect between H_1 and H_{20} indicated an equatorial position for C_{20}. NOE effect between H_{11} and H_{17} showed an equatorial position for C_{17}, and coupling constants are those of an axial H_6. If the C_1–C_{10} bond is axial, then the C_5–C_4 bond should be equatorial, and C_5–C_{18} is axial, anti to H_6. This was confirmed by the NOE effect between H_{11} and H_{19}. The assignment of the stereochemistry at C_2 was made difficult by the overlapping of the signals of the two H_1, and the lack of significant NOE effect for H_2. However, H_2 and C_2 displayed the same chemical shifts in compounds **3** and **1**, and were different from those of compound **2**. So we assumed that the relative stereochemistry at C_2 was the same in both compounds **3** and **1**. Laetiaprocerine B **4** and Laetiaprocerine C **5** showed an ambiguous NOE effect between H_1 and H_{20}. However, the axial position of the unsaturated side chain at C_9 could be deduced from the NOE effect between H_{11} and H_{19}. One H_1 correlated with the axial H_6 and H_2 indicating the same stereochemistry at C_2 as in compound **1**. Laetiaprocerine D **6** showed NOESY correlations allowing stereochemistry assignment at C_2: there was a NOE effect between H_{1a} and H_6, so H_6 is axial (this was not obvious from the coupling constants) and ring B could be considered as a pseudo chair. Both H_1 showed NOESY correlation with H_2, which could therefore be assigned as pseudo equatorial. The other NOE effects of **6** were comparable to those of **3**.

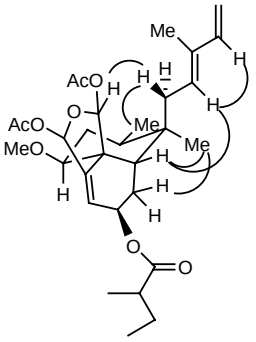

Figure 2. Key NOESY for Laetiaprocerine A.

We also tried to determine the absolute configuration of Casearlucine A **1** and Caseamembrol A **2**, which remained unknown. We used the modified Mosher's ester method described by Latypov et al.[16] and previously used by Prakash et al. on similar clerodane diterpenoids.[9] Acylation of **1** and **2** with (*R*)-MPA yielded the *R*-diesters **1*R*** and **2*R***, and acylation with (*S*)-MPA gave the *S*-diesters **1*S*** and **2*S***. The $\Delta\delta^{RS}$ obtained are summarized in Table 3. The results were similar to those obtained by Prakash et al. especially for H_{18}, but we disagree with their conclusions on the *R* absolute configuration at C_6. On the molecular models of **1*R*** and **1*S***, when the aryl ester moiety adopts the conformation described in the literature as the most stable,[16] H_{18} should be shielded in compound **1*S*** only if C_6 is *S* (Fig. 3). This absolute stereochemistry obtained for Casearlucine A and Caseamembrol A is identical to that obtained by Beutler et al. by X-ray crystallography (anomalous dispersion) for a similar clerodane diterpenoid, Casearborin E.[7] It is also identical to that obtained for Casearin C by chemical derivatization and circular dichroism spectroscopy by Itokawa et al.[11] This experiment confirmed that H_6 was axial on **1**: the coupling constants for H_6 on **1*R*** and **1*S*** were clearly those of an axial proton, which was not obvious on the ^{1}H NMR of **1**. Moreover, the ^{1}H NMR spectrum of each of the four acylation reaction crude extracts showed only one diastereoisomer, which tended to prove that **1** and **2** were optically pure. The absolute stereochemistry of Laetiaprocerine C **5** was determined by chemical correlation: benzoylation of **1** gave a compound identical to **5** (TLC, ^{1}H NMR, optical rotation) and thus both compounds have the same absolute configuration.

The structure of the new butanolide **7** ($C_{21}H_{38}O_3$, HREIMS) was established as follows. Spectral data of this compound were compared to those of the (2*R*,3*S*,4*S*)-3-hydroxy-4-methyl-2-(1′-*n*-hexadec-7′(*Z*)-enyl)butanolide isolated from *Trichilia claussenii*.[17] ^{1}H, ^{13}C NMR spectra were slightly different, and NMR signal assignment was made as follows: signals at 2.55, 4.20, and 4.65 ppm showed HMBC correlation with the carbonyl at 177.7 ppm. On the COSY

Table 3. δ and $\Delta\delta^{RS}$ values for the (R) and (S) MPA esters **1*R***, **1*S***, **2*R*** and **2*S***[a]

	δ **1*R***	δ **1*S***	$\Delta\delta^{RS}$**1**	δ **2*R***	δ **2*S***	$\Delta\delta^{RS}$**2**
H_{18}	6.59	6.00	0.59	6.57	6.02	0.55
H_{19}	6.48	6.59	−0.11	6.42	6.55	−0.13
H_3	6.01	5.83	0.18	5.90	5.73	0.17
H_2	5.44	5.35	0.09	5.60	5.54	0.06

[a] All spectra were recorded in $CDCl_3$, 250 MHz.

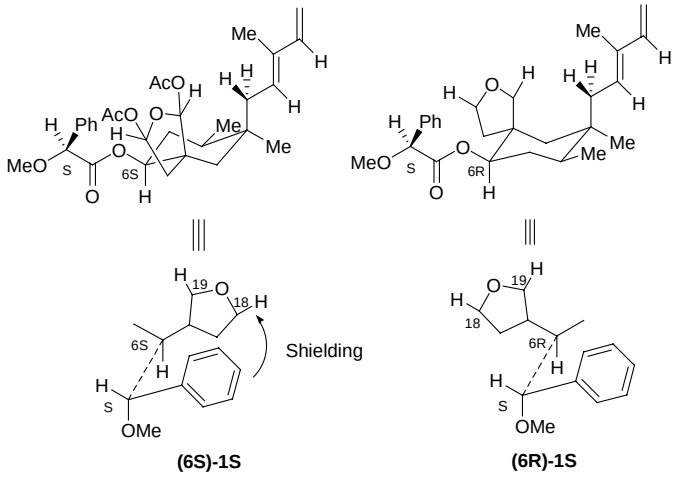

Figure 3. Comparison of shielding effect on H_{18} for (6*S*)-1*S* and (6*R*)-1*S* (partial structures).

spectrum, the signal at 4.20 ppm correlated with 2.55 ppm and 4.65 ppm. The signal at 4.65 ppm also correlated with a methyl doublet at 1.42 ppm. Signals at 2.55, 4.20, 4.65, and 1.42 ppm could be assigned to H_2, H_3, H_4, and Me-4, respectively. H_2 correlated with signals at 1.58 and 1.75 ppm, carried by a carbon at 28.6 ppm assigned to $H_{1'}$ and $C_{1'}$. This carbon is the first of an unsaturated fatty side chain. The position of the double bond on the chain was given by DMDS derivatization.[18] EI mass spectrum of the DMDS adduct gave a molecular pic at 432, and two fragment ions at 259 and 173. The coupling constant (10.8 Hz) between the two ethylenic protons was measured, while the allylic proton signal at 2.03 ppm was suppressed by irradiation, thus allowing us to assign the *Z* configuration for this double bond. The relative stereochemistry of the lactone ring was found by applying the rule described by Chaves and Roque:[19] $C_{1'}$ around 27 ppm indicated a fatty side chain *trans* to the hydroxyl at C_3, and $C_{Me\text{-}4}$ around 13 ppm indicated a methyl *cis* to the the same hydroxyl. This was confirmed by the NOESY spectrum, indicating NOE effects between Me-4 and H_2, H_3 and H_4, and H_3 and $H_{1'}$.

The clerodane diterpenoids exhibited mild antiplasmodial, leishmanicidal and cytotoxic activities when tested in vitro and the butanolide could be considered as inactive (Table 4).[20] Our compounds displayed antimalarial and cytotoxic activities close to activities previously reported for similar compounds.[3,5] Compounds **1**, **2**, and **3** were more active than **4**, **5**, and **6**, so bulky substituents on C_6, and the hydrolysis of the diacetal lowered biological activity. The effect of bulky substituents on C_6 was striking for the leishmanicidal activity: the most efficient compounds against *L. amanozensis* (IC_{50} around 10 μM) were **1** and **2**, while **4** and **5** were inactive. In compounds **4** and **5**, the benzoic ester at C_6 led to steric hindrance around C_{18} and C_{19} which are two electrophilic centers. Therefore, nucleophilic attack on C_{18} or C_{19} might be responsible for the antileishmanial activity of such compounds. The antiplasmodial activities of the diastereoisomers **1** and **2** were equivalent and similar to that of **3**, while the latter was less cytotoxic. The ether substitution on C_6 could explain this difference. However, none of these compounds seemed to have specific antiparasitic activity, but they should be good candidates for further investigation as cytotoxic agents.

Table 4. Biological activities (μM) of the clerodane diterpenoids **1**–**6** and the butanolide **7**[20]

	P. falciparum		*L. amazonensis*		Human cells	CAR[a]
	F-32 (2)[b]	FcB1 (3)	Axenic amastigotes (2)	Promastigotes (2)	MCF7 (3)	
1	0.62 ± 0.03[c]	0.54 ± 0.05	5.98 ± 6.8	11.1 ± 0.2	1.54 ± 0.88	2.2
2	0.57 ± 0.04	0.59 ± 0.02	10.5 ± 0.4	11.0 ± 0.2	0.85 ± 0.21	1.3
3	0.58 ± 0.03	0.66 ± 0.08	47.4 ± 29.8	10.9 ± 0.1	4.38 ± 0.29	6.8
4	4.44 ± 0.46	6.08 ± 1.46	> 200	> 200	17.8 ± 1.71	3.1
5	4.66 ± 0.23	5.35 ± 0.94	> 200	> 200	27.3 ± 4.25	4.6
6	6.04 ± 0.66	3.79 ± 0.71	30.3 ± 0.5	50.9 ± 37.6	9.60 ± 2.16	2.7
7	57.6 ± 10.4	27.5 ± 4.51	129 ± 7.1	111 ± 34.7	65.9 ± 32.4	2.1
CQ[d]	60×10^{-3}	145×10^{-3}	ND	ND	ND	ND
AmB[e]	ND	ND	0.3	0.3	ND	ND
Dox[f]	ND	ND	ND	ND	0.4	ND

[a] CAR cytotoxic/antiplasmodial (FcB1) ratio.
[b] Number of independent experiments.
[c] Means ± SD.
[d] CQ, chloroquine; positive control for *P. falciparum* inhibition.
[e] AmB, amphotericin B; positive control for *Leishmania* inhibition.
[f] Dox, doxorubicin; positive control for MCF7 inhibition.

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14. We worked on the bark of two different trees. Interestingly, one tree (N° 424, diameter 23 cm, collected in January 2003) gave compounds **1** and **2** as the major compounds, with small amounts of **3** and **7**, whereas the other (N° 1003, diameter 43 cm collected in March 2003) gave **3** and **7** as the main compounds, with small amounts of **1**, **4**, **5** and **6**.
15. Structural data. Laetiaprocerine A (**3**): $[\alpha]_D$ +48.5° (*c* 0.33, MeOH); HRTOFESIMS *m/z* 555.2939 (MNa^+, calcd for $C_{30}H_{44}O_8Na$: 555.2934, +0.9 ppm); IR (KBr) 2970, 2937, 2879, 1754, 1730, 1460, 1373, 1230. Laetiaprocerine B (**4**): $[\alpha]_D$ +133° (*c* 0.40, MeOH); HRTOFESIMS *m/z* 631.2874 (MNa^+, calcd for $C_{35}H_{44}O_9Na$: 631.2883, −1.4 ppm) ; IR (KBr) 2966, 2928, 2870, 1752, 1719, 1451, 1371, 1272, 1254, 1224. Laetiaprocerine C (**5**) $[\alpha]_D$ +89° (*c* 0.33, MeOH); HRTOFESIMS *m/z* 645.3015 (MNa^+, calcd for $C_{36}H_{46}O_9Na$: 645.3040, −3.9 ppm); IR (KBr) 2969, 2937, 2878, 1757, 1726, 1452, 1372, 1273, 1226. Laetiaprocerine D (**6**) $[\alpha]_D$ +102° (*c* 0.43, MeOH); HRTOFESIMS *m/z* 453.2613 (MNa^+, calcd for $C_{26}H_{38}O_5Na$: 453.2617, −0.8 ppm); IR (KBr) 2969, 2935, 2878, 1730, 1638, 1460, 1376. Laetianolide A (**7**): $[\alpha]_D$ −32° (*c* 0.58, MeOH); HREIMS *m/z* 338.28219 (M^+, calcd for $C_{21}H_{38}O_3$: 338.28209, +0.3 ppm); IR (KBr) 2925, 2854, 1756, 1464, 1340, 1189; ^1H NMR ($CDCl_3$, 400 MHz) 5.36 (2H, m, ethylenic); 4.64 (1H, m, H_4); 4.22 (1H, dd, J = 4.6–3.8 Hz, H_3); 2.55 (1H, m, H_2); 2.03 (4H, m, allylic); 1.75 (1H, m, $H_{1'}$); 1.61 (1H, m, $H_{1'}$); 1.50 (2H, m, CH_2 fatty chain); 1.42 (3H, d, J = 6.6 Hz, Me-4); 1.40–1.25 (18H, m, CH_2 fatty chain); 0.90 (3H, t, J = 7.0 Hz, CH_3 fatty chain). ^{13}C NMR($CDCl_3$, 100 MHz) 177.7 (C_1); 130.3–129.8 (ethylenics); 78.3 (C_4); 74.4 (C_3); 49.4(C_2); 32.1–29.9–29.8–29.7–29.5–29.2 (CH_2, fatty chain); 28.6 ($C_{1'}$); 27.4 (allylic); 22.9 (CH_2 fatty chain); 14.3 (Me-4); 14.1 (CH_3 fatty chain).
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20. *Plasmodium falciparum* was cultured according to the method described by Trager and Jensen,[20a] with modifications.[20b] Cultures were synchronized by 5% D-sorbitol lysis (Merck, Darmstadt, Germany).[20b] F32 Tanzania was considered as a chloroquino-sensitive strain (chloroquine IC_{50}: 60 ± 12 nM, <100 nM), FcB1-Columbia was considered as a chloroquino-resistant strain (chloroquine IC_{50}: 145 ± 11.2 nM). In vitro antimalarial activity was evaluated by [^{3}H]hypoxanthine (ICN, France) incorporation as already described by Desjardins et al.[20c] Incubation time between parasite culture and the drugs was 48 h. The cytotoxicity of the drugs was estimated on human breast cancer cells (MCF7). Cell lines were cultured in the same conditions as *P. falciparum*, except for the 5% human serum which was replaced by 5% fetal calf serum (Boehringer). After addition of drugs at various concentrations, cell growth was estimated by [^{3}H]hypoxanthine incorporation after 48 h incubation.[20d] The antileishmanial (*Leishmania amazonensis*) activity against promastigote and axenic amastigote was determined after 72 h incubation by a colorimetric method based on the reduction of tetrazolium salt (MTT, Sigma)[20e] (a) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673; (b) Benoit, F.; Valentin, A.; Pélissier, Y.; Marion, C.; Dakuyo, Z.; Mallié, M.; Bastide, J.-M. *Trans. Roy. Soc. Trop. Med. Hyg.* **1995**, *89*, 217; (c) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710; (d) Valentin, A.; Benoit-Vical, F.; Moulis, C.; Stanislas, E.; Mallié, M.; Fourasté, I.; Bastide, J.-M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2305; (e) Sereno, D.; Lemesre, J.-L. *Antimicrob. Agents Chemother.* **1997**, *41*, 972.