Phytochemistry 70 (2009) 305–311

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Four anti-protozoal and anti-bacterial compounds from *Tapirira guianensis*

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ARTICLE INFO

Article history:
Received 21 February 2007
Received in revised form 1 October 2008
Available online 29 November 2008

Keywords:
Tapirira guianensis Aubl
Anacardiaceae
Cyclic alkyl polyol derivatives
Anti-plasmodial
Anti-leishmanial
Anti-bacterial
Plasmodium falciparum
Leishmania amazonensis

ABSTRACT

Tapirira guianensis is a common tree used in traditional medicine in French Guiana against several infectious diseases (malaria, leishmaniasis, bacteria, etc.). The bioassay-guided purification of CH_2Cl_2 bark extract led to the isolation of four cyclic alkyl polyol derivatives: 4,6,2′-trihydroxy-6-[10′(Z)-heptadecenyl]-1-cyclohexen-2-one **(1a)**, 1,4,6-trihydroxy-1,2′-epoxy-6-[10′(*Z*)-heptadecenyl]-2-cyclohexene **(1b)**, 1,4,5,2′-tetrahydroxy-1-[10′(Z)-heptadecenyl]-2-cyclohexene **(2)**, and 1,3,4,6-tetrahydroxy-1,2′-epoxy-6-[10′(*Z*)-heptadecenyl]-cyclohexane **(3)**. The structures were established on the basis of 1D and 2D NMR analyses. The anti-leishmanial, anti-plasmodial, anti-bacterial (on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*), and anti-fungal (on *Candida albicans*) activities of the extracts and of these original compounds were evaluated. Two showed medicinal interest supporting the traditional uses of the plant. The structures were established through spectral analyses of the isolates and their derivatives.

1. Introduction

The genus *Tapirira,* which is composed of approximately 35 species, belongs to the Anacardiaceae family and is found in Mexico and throughout South America. *Tapirira guianensis* Aubl. is a tall tree, occurring in the Amazon region and used in traditional medicine. The tree is commonly named "Monben-fou" by the creoles or "Ara" by the Palikur indians. Its bark is often used as a decoction against malaria and diarrhoea (Deharo et al., 2001). In French Guiana, the sap is applied against thrush, external infected wounds (fungi or bacteria) and is also used as in other Anacardiaceae against cutaneous leishmaniasis (Grenand et al., 1987). The literature reports studies of the biological activities of the alcoholic extract of the dried plant as a cytotoxic (Suffness et al., 1988) or as a uterine stimulant (Barros et al., 1970). The $CHCl_3$ extract of the seeds are used for its anti-cancer activity. Phytochemical studies report the isolation of β-sitosterol and two alkylphenols from the MeOH extract of the seeds (David et al., 1998; Correia et al., 2001). Previous chemical investigations of the hexane extract of the bark of *T. guianensis* led to the isolation of three triterpenoids, six alkyl ferulates (Correia et al., 2003) and two cyclohexenones (Hoarau and Pettus, 2006). We report here the identification of four new cyclic alkyl polyol derivatives isolated from the CH_2Cl_2 extract of the bark of *T. guianensis* with their anti-leishmanial, antiplasmodial and bactericidal properties.

2. Results and discussion

2.1. Identification of compounds

The highest antiplasmodial activity was found in the dichloromethane extract of the bark of *T. guianensis* Aubl. (IC_{50} F32: 18.0 μg/ml) in comparison with aqueous, methanol and hexane extracts which were inactive. The bioassay-guided purification of the CH_2Cl_2 extract led to the identification of four new compounds (**1a**, **1b**, **2** and **3**). NMR analyses were performed in various solvents but total assignments were established in DMSO-d_6 (Table 2) except for NOESY and ROESY experiments for which $CDCl_3$ or CD_3OD (Table 3, Fig. 2) were used to avoid interfering signals from the OH functions of the test molecules. Compounds **1a** and **1b** were obtained in a mixture as a colourless oil with the same molecular formula $C_{23}H_{40}O_4$ determined from its ESI-Q-TOF-HRMS at *m/z* 381.302 $[M+H]^+$ (Δ ppm: 3.9 ppm). The IR spectrum showed a broad OH absorption band at 3373 cm^{-1} and absorption of a conjugated carbonyl at 1681 cm^{-1}. Although **1a**–**1b** appeared to

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doi:10.1016/j.phytochem.2008.10.003

be homogenous by TLC and HPLC, close examination of its ^{1}H and ^{13}C NMR data (firstly recorded in $CDCl_3$) suggested that it was still a mixture as there was slight doubling up of certain signals, particularly in the ^{13}C NMR spectrum, presenting 35 peaks. The hypothesis of the presence of different optical isomers in this mixture was rejected because of changes in the apparent peak intensity ratios when NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 (**1a**/**1b** = 1/2 in DMSO-d_6 and **1a**/**1b** = 1/1 in $CDCl_3$) or when the spectra where recorded at higher temperatures (at 323 K: 60.4% of **1a** and 39.6% of **1b** and at 303 K 45.5% of **1a** and 54.5% of **1b** in $CDCl_3$). Thus, the presence of two forms in equilibrium generated in solution was suggested. Similar correlations for compounds **1a** and **1b** on the 2D NMR spectra (HMBC) between numerous aliphatic carbons (δ_C 25.5–40.2) with common olefinic protons (δ_H 5.32–5.34) (4H, *t*, *J* = 5.0 Hz) indicated the presence of a long mono-unsaturated alkyl chain (Tables 1 and 2). Close examination of the 2D NMR spectra (*i.e.* HSQC, HMBC and ^{1}H–^{1}H COSY) of the mixture, allowed us to distinguish the two forms **1a** and **1b**. Compound **1a** showed signals characteristic of an α,β-unsaturated ketone (carbonyl at δ_C 198.7, two coupling olefinic protons at δ_H 5.78 and 6.85), as well as two secondary alcohols at δ_C 63.7 and 66.8 (coupling, in the HSQC experiment, to the protons at δ_H 4.49 and the AB system 3.72–4.37, respectively), and a tertiary alcohol at δ_C 73.4. Besides these functional groups, there was a signal originating in a methylene carbon at δ_C 44.0 coupling in the HSQC spectrum with two protons at δ_H 1.80 and 2.39. The HMBC cross peaks of these protons with the carbons corresponding to the carbonyl, an olefin and the secondary and tertiary alcohols allowed us to assume a dihydrocyclohexenone moiety for **1a** substituted at C-6 with a 17-carbon mono-unsaturated alkyl chain. These observations are in agreement with the molecular formula $C_{23}H_{40}O_4$ and the chemotaxonomy of Anacardiaceae where this kind of compound has already been described (Queiroz et al., 2003). The hydroxyl (δ_C 66.8) group was located at C-2′ on the basis of its ^{1}H–^{1}H COSY and HMBC correlations with the methylenes of the alkyl chain and carbon C-6 (δ_C 73.4). The proposed structure of the

Table 1
^{13}C NMR (DMSO-d_6, $CDCl_3$ or CD_3OD) data of compounds **1a**, **1b**, **2** and **3.**

C	1a		1b		2		3	
	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	CD_3OD
1	198.7	201.8	98.1	98.1	73.3	73.5	101.4	101.9
2	126.1	125.8	127.6	126.4	132.7	132.5	37.2	37.6
3	153.9	149.6	133.3	133.8	129.3	129.3	69.2	68.8
4	63.7	63.6	63.7	64.6	70.3	70.3	67.4	70.9
5	44.0	40.3	41.8	40.5	63.9	65.3	37.5	37.2
6	73.4	74.2	78.5	79.0	42.5	42.3	77.7	79.3
1′	43.6	44.5	44.3	44.3	46.2	45.8	43.5	44.4
2′	66.8	69.1	75.8	75.8	66.2	68.2	75.0	77.5
3′	39.2	40.2	38.0	38.0	38.5	38.6	38.1	39.3
4′–8′	25.5–29.7	25.1–29.7	25.5–29.7	25.5–29.7	25.1–29.2	25.1–29.2	26.0–29.6	30.1–30.9
9′	27.0	27.2	27.0	27.2	26.6	27.3	27.1	28.2
10′–11′	130.1	130.2	130.1	130.2	129.6	129.8	130.2	130.9
12′	27.0	27.2	27.0	27.2	26.6	27.3	27.1	28.2
13′–14′	25.5 and 29.7	25.5 and 29.7	25.5 and 29.7	25.5 and 29.7	28.3 and 29.6	28.3 and 29.6	28.7 and 29.6	30.1 and 30.9
15′	31.6	31.6	31.6	31.6	31.2	31.6	31.6	27.1
16′	22.6	22.6	22.6	22.6	22.1	22.1	22.6	23.8
17′	14.4	14.4	14.4	14.4	14.1	14.1	14.4	15.1

Table 2
^{1}H NMR (DMSO-d_6) data of compounds **1a**, **1b**, **2** and **3**, δ_H ppm, *J* (Hz).

H	1a	1b	2	3
2	5.78 *dd* (10.2, 2.0)	5.49 *dd* (10.2, 2.0)	5.62 *d* (10.3)	1.65 *d* (14.5, H_{ax}) 1.97 *d* (14.5, H_{eq})
3	6.85 *dt* (10.2, 2.3, 1.2)	5.59 *dt* (10.2, 1.4)	5.36 *dt* (10.3, 1.8)	3.61 *m*
4	4.49 *m*	4.23 *m*	3.81 *m*	3.64 *dd* (10.6, 4.1)
5	1.80 *m*, H_{ax} 2.39 *ddd* (13.6, 5.0, 1.4, H_{eq})	1.55 *m*, H_{ax} 1.94 *dd* (13.4, 6.0, H_{eq})	4.20 *m*	1.53 *dd* (13.5, 4.1, H_{eq}) 1.77 *dd* (13.5, 10.6, H_{ax})
6	–	–	1.29 *m*, H_{eq} 2.03 *dd* (13.2, 5.2, H_{ax})	–
1′	1.60 *dd* (14.7, 9.7) 1.78 *dd* (14.7, 2.4)	eq 1.82 *m*/ax 1.89 *m*	1.42 *d* (14.5) 1.62 *dd* (14.5-10.2)	1.72 *d* (3.3 H_{eq}) 1.95 *m*, H_{ax}
2′	3.72 *m*	3.80 *m*	3.78 *m*	3.82 *m*
3′	1.40-1.51 *m*	1.42 *m*/1.55 *m*	1.39-1.53 *m*	1.42 *m*/1.59 *m*
4′–8′	1.21 *m*/1.35 *m*	1.21 *m*/1.35 *m*	1.20 *m*/1.36 *m*	1.21 *m*/1.33 *m*
9′	1.99 *m*	1.99 *m*	1.98 *m*	1.99 *m*
10′-11′	5.34 *t* (5.0)	5.34 *t* (5.0)	5.32 *t* (4.7)	5.34 *t* (4.7)
12′	1.99 *m*	1.99 *m*	1.98 *m*	1.99 *m*
13′-16′	1.21 *m*/1.35 *m*	1.21 *m*/1.35 *m*	1.20 *m*/1.36 *m*	1.21 *m*/1.33 *m*
17′	0.86 *t* (7.1)	0.86 *t* (7.1)	0.85 *t* (7.0)	0.87 *t* (7.0)
OH-1	–	5.93 *s*	4.95 *s*	5.65 *s*
OH-3	–	–	–	3.61 *m*
OH-4	4.73 *d* (5.5)	4.73 *d* (5.5)	4.20 *m*	4.24 *d* (5.7)
OH-5	–	–	4.60 *d* (5.8)	–
OH-6	5.35 *s*	4.20 *s*	–	4.27 *s*
OH-2′	4.37 *d* (6.0)	–	4.94 *m*	–

ax: Pseudo-axial or axial; eq: pseudo-equatorial or equatorial.

second form, **1b**, was determined from a characteristic ^{13}C NMR signal with a quaternary carbon at δ_C 98.1, whose HMBC connectivities were similar to those of the carbonyl for **1a** with a supplementary cross peak between the proton of the secondary alcohol at C-2′ (δ_C 75.8) and the hemiacetal carbon C-1 (δ_C 98.1). It is worth mentioning that the **1b** form seems to be generated in solution and we suggest that it derives from an intramolecular nucleophilic attack by the alcohol located on C-2′ (δ_C 66.8) on the α,β-unsaturated ketone resulting in a five-membered ring with an α,β-unsaturated hemiacetal as drawn for **1b** (Fig. 1). This was supported by the different ratios for the two forms according to the solvent used for NMR experiments and to the temperature of analysis. The position of the double bond in the alkyl side chains of **1a** and **1b** was determined from the EI-MS fragments of the α,β-*bis*-(methylthio)-derivatives produced by the reaction of mixture **1a**–**1b** with dimethyl disulfide (Mansour et al., 2005). The EI-MS spectrum of the thiomethylated derivatives showed an intense fragment ion at *m/z* 145 corresponding to the cleavage of $[C_8H_{17}S]^+$. The presence of this fragment clearly revealed that the double bond, on the alkyl side chain, was originally located between the seventh and eighth carbons from the terminal methyl group, namely at C10′–C11′. The specific chemical shift of the methylene groups adjacent to an aliphatic double bond system ($\delta_{C\text{-}12 \text{ and } C\text{-}9}$. 27.0 ppm) established the *Z*-configuration of this double bound in accordance with previous studies on lipid chains (Lie Ken Jie et al., 1997) or alkyl phenol compounds (Correia et al., 2001) which indicated a higher value for the *E* configuration (δ_C 32.0 ppm). The relative configurations of **1a**–**1b**, were established on the basis of coupling constants (recorded in DMSO-d_6) and NOE experiments (recorded in $CDCl_3$) allowing the connectivities of the non-exchangeable protons to be observed (Fig. 2). The rigidity of the cyclohexene moiety permitted a clear interpretation of the coupling constants. Thus, the *J*-based values for H-5 considered as equatorial protons for **1a** and **1b** (δ_H 2.39 and 1.94, respectively) were around 5 Hz, revealing the pseudo-axial disposition of H-4 (**1a** δ_H 4.49 and **1b** δ_H 4.23) and by inference, the pseudo-equatorial position of OH-4 (δ_H 4.73). Key NOE correlations (in $CDCl_3$) for **1a** were observed between H-4 (δ_H 4.59) and H-5eq (δ_H 2.53), and H-5eq with the first protons of the alkyl chain ($H_{1'}$, δ_H 1.70 and 1.99) suggesting the pseudo-equatorial position for OH-6. Concerning the relative configuration of **1b**, the NOE cross peaks between H-4ax (δ_H 4.53) and H-5eq (δ_H 2.26) confirm the previously deduced protons disposition. On the other hand NOESY or ROESY cross peaks and *J*-based values observed for the configuration of the ring junction cycle are ambiguous and contradictory. The data could be explained if the intramolecular cyclisation of **1a**–**1b** provided two kinds of configuration for **1b** at C-1 (Figs. 1 and 2). Therefore, the two forms **1a** and **1b** were, respectively identified as 4,6,2′-trihydroxy-6-[10′(*Z*)-heptadecenyl]-2-cyclohexenone and 1,4,6-trihydroxy-1,2′-epoxy-6-[10′(*Z*)-heptadecenyl]-2-cyclohexene. The ESI-Q-TOF-HRMS of compound **2** showed a pseudomolecular ion peak at *m/z* 383.313 $[M+H]^+$ (calculated for $C_{23}H_{43}O_4$ 383.3161). This result, combined with ^1H and ^{13}C NMR spectra (similar to those of **1a**), showed that the structure of **2** was in good agreement with a hydroxylated cyclohexene moiety substituted by a 17-carbon mono-unsaturated alkyl chain. The ^{13}C NMR spectrum (in DMSO-d_6) revealed characteristic signals of four hydroxyl groups (one tertiary alcohol at δ_C 73.3; three secondary alcohols at δ_C 70.3, 63.9 and 66.2) and two double bonds (four methine carbons between δ_C 129.3 and 132.7 ppm). The correlations between the two olefinic protons H-2 (δ_H 5.62) and H-3 (δ_H 5.36) with one tertiary (C-1) and two secondary alcohols (C-4, C-5) observed in the HMBC spectrum, suggested the presence of a trihydroxycyclohexene moiety. In the same way, the position of the last hydroxyl group at C-2′ (δ_C 66.2) and the substitution of the alkyl chain at C-1 (δ_C 73.3), was determined by the cross peak observed, in the HMBC spectrum, between the quaternary carbon at δ_C 73.3 (C-1) and the proton at δ_H 3.78 (H-2′). As for **1a** and **1b**, the double bond of the alkyl side chain was located between C-10′ and C-11′ on the basis of an intense signal at *m/z* 145 observed in the EI-MS spectrum of the α,β-*bis*-(methylthio)-derivative of compound **2.** The *Z*-configuration was deduced from the "low" chemical shift of the methyl groups adjacent to the diene system ($\delta_{C\text{-}12 \text{ and } C\text{-}9}$ 26.6). The relative configuration of carbons C-1, C-4 and C-5 was determined from the coupling con-

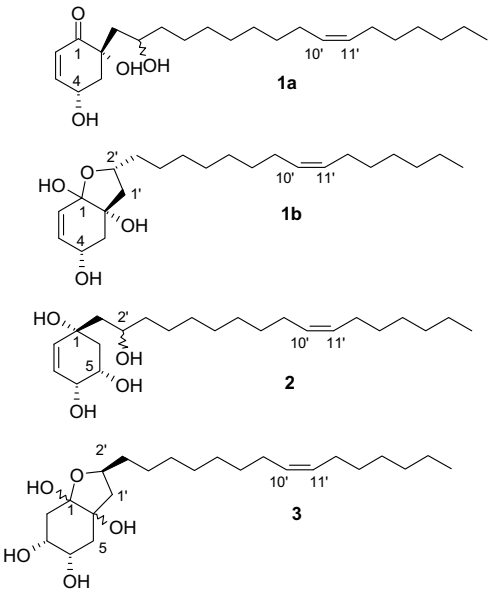

Fig. 1. Relative configuration of the isolated compounds.

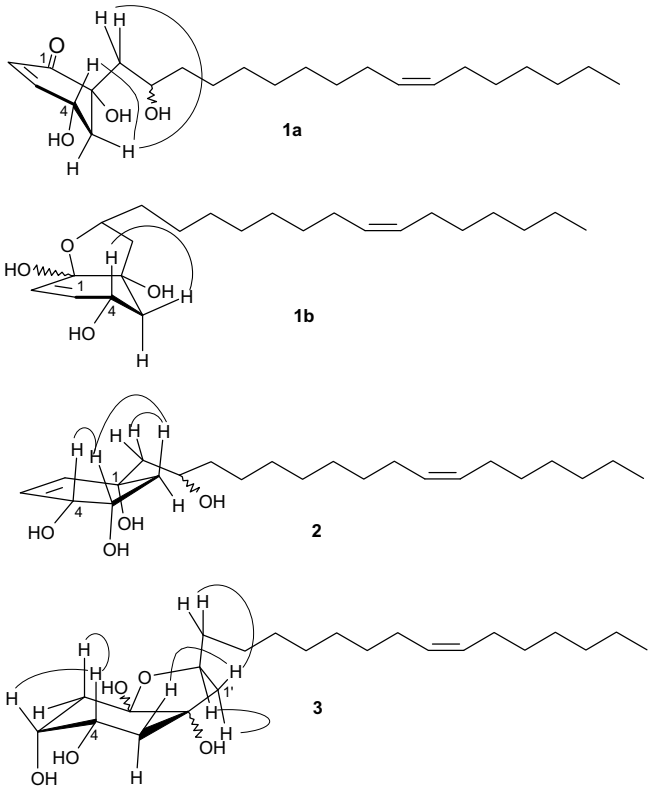

Fig. 2. Selected NOESY and ROESY correlations observed for compounds **1a**, **1b**, **2** ($CDCl_3$) and **3** (in CD_3OD).

Table 3
^{1}H NMR data of compounds **1a**, **1b**, **2** ($CDCl_3$) and **3** (CD_3OD), δ_H ppm, *J* (Hz).

H	1a	1b	2	3
2	6.05 *dd* (10.1, 0.5)	5.64 *dd* (10.2, 2.0)	5.87 *dd* (10.3, 1.6)	1.73 *m*, H_{ax} 1.89 *m*, H_{eq}
3	6.86 *ddd* (10.1, 4.2, 1.2)	5.83 *dd* (10.2, 1.4)	5.61 *dt* (10.3, 2.1)	3.82 *m*
4	4.59 *q* (8.2, 4.2)	4.53, *m*	4.03 *dd* (2.2, 4.3)	3.77 *m*
5	2.24 *m*, H_{ax}	1.69, *m*, H_{ax}	4.54 *m*	1.83 *dd* (14.8 3.8, H_{eq})
	2.53 *ddd* (14.5, 3.4, 1.3, H_{eq})	2.26 *ddd* (12.8, 5.2, 1.2 H_{eq})		2.13 *dd* (14.8, 3.2, H_{ax})
6			1.43 *m*, H_{eq} 2.42 *dd* (13.3, 4.9, H_{ax})	
1′	1.70 *m*	1.90 *dd* (12.6, 9.7)	1.57 *dd* (14.8, 1.34)	1.87, *m*, H_{eq}
	1.99 *m*	2.11 *m*	1.88 *dd* (14.8, 10.8)	1.97, *m*, H_{ax}
2′	4.10 *m*	3.99 *m*	4.09 *m*	3.96 qd (8.8, 6.8)
3′	1.42–1.54 *m*	1.50 *m* 1.66 *m*	1.45–1.55 *m*	1.46 *m* 1.67 *m*
4′–8′	1.22–1.33 *m*	1.2–1.33 *m*	1.20–1.36 *m*	1.21–1.33 *m*
9′	2.05 *m*	2.05 *m*	2.03 *m*	1.99 *m*
10′–11′	5.32 *m*	5.32 *m*	5.37 *m*	5.30 *m*
12′	2.05 *m*	2.05 *m*	2.03 *m*	1.99 *m*
13′–16′	1.22–1.33 *m*	1.22–1.33 *m*	1.20–1.36 *m*	1.21–1.33 *m*
17′	0.86 *t* (7.0)	0.86 *t* (7.0)	0.85 *t* (7.0)	0.92 *t* (7.1)

stant of one of the H-6 protons and from the NOESY and ROESY results (Fig. 2). A 5.2 Hz value for the coupling constant of the pseudo-axial H-6 proton suggested the pseudo-equatorial orientation for H-5 (so, OH-5 is pseudo-axially oriented). This was supported by NOE correlations (in $CDCl_3$, Table 3) observed between H-5 pseudo-equatorial (δ_H 4.54) with H-6 pseudo-axial (δ_H 2.42) and pseudo-axial H-4 (δ_H 4.03). The NOE cross peaks observed between pseudo-axial H-6 (δ_H 2.42) and methylene protons from the alkyl chain (H-1′, δ_H 1.57 and 1.88) allowed us to deduce that H-4, H-5, H-6ax and H-1′ were oriented on the same side of the cyclohexene ring. Compound **2** was identified as 1,4,5,2′-tetrahydroxy-1-[10′(*Z*)-heptadecenyl]-2-cyclohexene. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compound **3** were very similar to those observed for compound **1b**. The differences consisted in the presence of one more hydroxyl group and only one double bond (instead of two for **1b**). These observations were supported by the ESI-Q-TOF-HRMS data of **3** indicating a molecular formula of $C_{23}H_{42}O_5$ (*m/z* 399.307, $M+H^+$), corresponding to 18 mass units greater than **1b**. The HMBC spectrum (in DMSO-d_6) allowed us to easily locate the new hydroxyl group at C-3 (δ_C 69.2) and to determine a cyclohexanol ring. The EI-MS spectrum of the α,β-*bis*-(methylthio)-derivative of **3** exhibited the same intense *m/z* 145 signal as the other compounds, indicating a similar position of the double bond on the chain. The relative configuration of the cyclohexanol moiety was established as above: an axial orientation was assumed for H-4 (δ_H 3.64) from its typical coupling constants of 10.6 Hz (*trans*-diaxial coupling) and 4.1 Hz (axial-equatorial coupling) with the two protons H-5 (δ_H 1.53 and 1.77). The NOESY and ROESY correlation (in CD_3OD) observed between H-4ax (δ_H 3.77) and H-3 (δ_H 3.82) revealed an equatorial disposition for H-3. The other strong NOE interactions observed between H-5eq (δ_H 1.83) and H-1′eq (δ_H 1.87) or H-1′eq with the protons of the alkyl chain H-3′ (δ_H 1.46–1.67) indicated a common orientation on the same side of the molecule for these protons. This hypothesis was confirmed by the NOE correlation of H-1′ax (1.97) with H-2′ (3.96) and suggested a relative configuration for **3** (Fig. 2). Relative configurations of two asymmetric carbons (C-1 and C-6) were not clearly defined because of the lack of resolution of superposed signals whatever the temperature or the solvent (DMSO-d_6, CD_2Cl_2 or CD_3OD). The structure of **3** was therefore established as 1,3,4,6-tetrahydroxy-1,2′-epoxy-6-[10′(*Z*)-heptadecenyl]-cyclohexane. It is worth mentioning that the structure of **3** is similar to **1b** but this compound was only present in a single form whatever the temperature or the solvent used for the analyses (DMSO-d_6, CD_3OD). We suggest that these differences of behaviour in solution may be due to the lack of a double bond in the cyclic moiety leading to a more stable chair conformation for **3**.

Compounds **1a**, **1b**, **2** and **3** were purified and identified here for the first time not only in *T. guianensis* but also in the plant kingdom.

2.2. Biological activities of the compounds

Extracts and isolated compounds were tested *in vitro* on parasitic protozoa (*Plasmodium falciparum* and *Leishmania amazonensis*), bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherishia coli*) and a fungus (*Candida albicans*) in accordance with the traditional uses of the plant. The tests revealed biological activities of the **1a**–**1b** mixture against *L. amazonensis*, *P. falciparum*, *S. aureus*, and *S. epidermidis* (Tables 4 and 5). The high activity (IC_{50}: 1.0 μM) and selectivity (C.A.R: 7) of the **1a**–**1b** mixture (main compounds in the extract) against *L. amazonensis* as well as its activity against *S. aureus* (IC_{50}: 75.4 μM) and *S. epidermidis* (IC_{50}: 17.6 μM) supported the traditional use of the plant against external infec-

Table 4
Antiparasitic activity of isolated compounds and CH_2Cl_2 bark extract.

Samples	*P. falciparum* (2)[b] IC_{50} (μM)		*L. amazonensis* (2) IC_{50} (μM)	Cytotoxicity (2) IC_{50} (μM)	C.A.R[a]		
	F32	FcB1	LMA cl1	MCF-7	F32	FcB1	LMAcl1
1a + 1b	4.7 ± 0.3[c]	5.4 ± 1.7	1.0 ± 0.1	6.8 ± 0.2	1.4	1.3	6.8
2	86.4 ± 2.6	92.9 ± 1.3	95.5 ± 22.2	34.8 ± 7.7	0.6	0.4	0.4
3	101.8 ± 3.8	159.5 ± 13.8	90.4 ± 7.5	74.1 ± 6.3	1.1	2.1	0.8
CH_2Cl_2 extract	18.0 ± 3.0 μg/ml	27.0 ± 1.0 μg/ml	38.5 ± 2.5 μg/ml	2.3 ± 0.3 μg/ml	0.7	0.1	0.1
CQ[d]	60×10^{-3}	0.14	n.d.	n.d.	n.d.	n.d.	n.d.
Dox[e]	n.d. [f]	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
AmB[g]	n.d.	n.d.	0.3	n.d.	n.d.	n.d.	n.d.

[a] CAR cytotoxic/anti-plasmodial or anti-leishmanial ratio.
[b] Number of independent experiments.
[c] Means ± SD.
[d] CQ: chloroquine;positive control for *P. falciparum* inhibition.
[e] Dox: doxorubicin; positive control for MCF7 inhibition.
[f] n.d.: not determined.
[g] AmB: amphotericine B, positive control for *Leishmania* inhibition.

Table 5
Anti-bacterial activity of isolated compounds and CH_2Cl_2 bark extract.

Samples	Bacteria (2)[a]					
Staphyloccocus	*S. aureus* IC_{50} (μM)	*S. aureus* BC[b] (μM)	*S. aureus* C.A.R.[c]	*S. epidermidis* IC_{50} (μM)	*S. epidermidis* BC (μM)	*S. epidermidis* C.A.R.
1a + 1b	75.4	150.8	11.0	17.6	35.2	0.1
2	n.a.[d]	n.a.	n.a.	n.a.	n.a.	n.a.
3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CH_2Cl_2 extract	250 μg/ml	500 μg/ml	108	250 μg/ml	500 μg/ml	0.0

[a] Number of independent experiments.
[b] BC: bactericidal concentration.
[c] CAR cytotoxic/IC_{50}.
[d] n.a.: not active.

tions, especially against cutaneous *Leishmania* and its *Staphylococcus* complications. A medicinal formulation based on a mixture of the bark powder or extract incorporated into a fatty vehicle (e.g. oil or wax) could be an interesting and accessible therapeutic alternative to classical medicines such as antimony derivatives and amphotericine B which have side effects or lead to resistance. The study also revealed an activity of **1a**–**1b** compounds against a chloroquine-sensitive strain (F32, IC_{50}: 4.7 μM) and a chloroquine-resistant strain (FcB1, IC_{50}: 5.4 μM) of *P. falciparum*. The antiplasmodial activity against the chloroquine-resistant strain was satisfactory but its lack of specificity (CAR: 1.3) and rather high toxicity (IC_{50} MCF7: 6.8 μM) prohibit long-term oral administration for malaria prophylaxis. The other compounds (**2** and **3**) did not show any interesting activity against the pathogens tested although they were chemically quite similar. These results show that it is the **1a**–**1b** mixture which is mainly responsible for the activities of the plant extract. Comparison of the different structures suggests that compound **1a** is responsible for the activity, because it is the only one containing a conjugated ketone, a Michael acceptor, often involved in pharmacological properties.

3. Conclusion

The traditional uses of the bark of *T. guianensis* against infectious diseases such as malaria, leishmaniasis and other bacterial or fungal diseases in French Guyana led us to select this species to carry out activity-guided purification of antiplasmodial compounds. The study permitted the identification of four new cyclic alkyl polyol derivatives including a mixture of two closely related molecules active against *P. falciparum* and, especially against cutaneous *Leishmania* and *Staphylococcus*. An external application of the active plant extract or of the purified compounds could represent an accessible therapeutic alternative to classical medicine against leishmaniasis.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a sodium lamp (λ = 589 nm) in a 10-cm microcell. IR spectroscopy (in KBr) was performed on a Perkin–Elmer Paragon 1000 FT-IR spectrophotometer. NMR spectra (500 MHz for ^1H NMR, and 125 MHz for ^{13}C NMR, both using TMS as internal standard) were obtained with DMSO-d_6, $CDCl_3$ or CD_3OD as solvents on a Bruker Avance II 500 spectrometer equipped with a TBI *z*-gradient 5 mm probe. 1D (^1H, ^{13}C) and 2D (COSY, HSQC, HMBC, NOESY, ROESY) NMR experiments were performed at 303 K using standard pulse sequences. Chemical shifts (δ) are given in ppm and coupling constants (*J*) are reported in Hz. EI-MS (70 eV) were recorded on a Thermoquest TSQ 7000 spectrometer, ESI-MS (3.5 kV in MeOH–CH_3CN) on a Perkin–Elmer Sciex API 365 mass spectrometer and HR-ESI-MS (positive-ion mode) on a Q-ToF Ultima (Waters) apparatus. Medium-pressure column chromatography was performed on silica gel 60 SDS (6–35 μm), silica gel C_{18} AIT (40–60 μm) and Mega Bond Elut Varian silica gel cartridges. Preparative TLC plates Merck 20 × 20 cm silica gel 60 F_{254}, 2 mm were impregnated with a solution of $AgNO_3$ (4 g in 4 ml of a 9/1 MeOH/H_2O mixture). Fractionation was monitored by TLC (silica gel 60 F-254, Merck) with visualization under UV (254 and 365 nm) and by spraying H_2SO_4-vanillin reagent. All solvents were spectral grade or distilled from glass prior to use.

4.2. Plant material

The bark of *T. guianensis* Aubl. (Anacardiaceae) was collected in French Guiana, in the vicinity of Cayenne in May 2005. Herbarium voucher specimens (GB 2853, GB 3051 and GB 2059) were deposited in the French Guiana Herbarium (CAY) and identified by Dr. G. Bourdy.

4.3. Extraction and isolation

After drying in a ventilated oven (48 h at 45 °C), the bark (2.6 kg) was pulverised and extracted three times with CH_2Cl_2 (5 l). The resulting dried extract (58 g) was defatted using a two-phase solvent system Hex/MeOH/CH_3CN (6:0.5:3.5) (4 l). The lower phase extract (35 g), which retains the biological activity, was submitted to medium-pressure column chromatography over silica gel (700 g) eluted with a gradient of CH_2Cl_2/MeOH (from 100% CH_2Cl_2 to 50% CH_2Cl_2/MeOH) (9 l). 72 fractions of 125 ml were recovered and then, on the basis of their TLC profile, combined into seven new fractions (F1 to F7). Fractions F4 (4 g) and F5 (7 g) displayed the best antiplasmodial activity (IC_{50} = 3 μg/ml). Fraction F5 was submitted to medium-pressure column chromatography on C_{18} silica gel eluted with various gradients of H_2O/CH_3CN (from 90:10 to 50:50) to afford the mixture **1a–1b** (956 mg). Fraction F4 was eluted on silica gel columns with different gradients of Tol/EtOAc (from 50% Tol/EtOAc to 100% Tol) and preparative TLC treated with $AgNO_3$ ($AgNO_3$–silica gel, 1:10) eluted with a mixture of Tol/EtOAc/MeOH (5:82:13). The final purifications were performed on silica gel medium-pressure columns eluted with Tol/EtOAc (35:65) to afford compound **2** (11 mg) and (25:75) to afford compound **3** (35 mg).

4.4. Biological activity tests

4.4.1. Plasmodium in vitro culture and antiplasmodial activity evaluation

Two *P. falciparum* strains were used: F32-Tanzania, considered as a chloroquine-sensitive strain (chloroquine IC_{50}: 60 nM) and

FcB1-Colombia considered as a chloroquine-resistant strain (chloroquine IC_{50}: 145 nM). Parasites were cultured according to the usual method (Trager and Jensen, 1976) with some modifications (Benoit et al., 1995). The cultures were synchronized every 48 h by 5% D-sorbitol lysis (Lambros and Vanderberg, 1979). *In vitro* antimalarial activity was analyzed by [^{3}H]-hypoxanthine incorporation as described (Desjardins et al., 1979) with modifications (Valentin et al., 1997). Inhibitions vs. concentration curves were plotted and the concentration inhibiting 50% of parasite growth was graphically determined. The 50% inhibitory concentration (IC_{50}) presented in the results sections is the mean (±SD) of three independent experiments. The positive control was chloroquine sulfate.

4.4.2. In vitro cytotoxicity evaluation

Cytotoxicity of the pure compounds was estimated on human breast cancer cells (MCF7). The cells were cultured in the same conditions as those used for *P. falciparum*, except for the 5% human serum, which was replaced by 5% fetal calf serum. For the determination of pure compound cytotoxicity, cells were distributed in 96-well plates at 2×10^4 cells/well in 100 µl, then 100 µl of culture medium containing extracts at various concentrations were added. Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after 48 h of incubation exactly as for the *P. falciparum* assay. The [^{3}H]-hypoxanthine incorporation in the presence of extracts or pure compounds was compared with that of control cultures without extract (positive control being doxorubicin). Fifty percent inhibitory concentrations were graphically determined from inhibition vs. concentration curves.

4.4.3. In vitro leishmanicidal activity

Leishmanicidal activity was evaluated against axenic amastigotes of *L. amazonensis*, a parasite responsible for cutaneous leishmaniasis in humans. Amastigotes were maintained *in vitro* as described (Sereno and Lemesre, 1997). They were then seeded (10^5 cells/ml, 100 µl/well) in 96-well plates. Growing dilutions of extracts or purified compounds were added and the plates were incubated at 30 °C (Jullian et al., 2005). After 48 h, [^{3}H]-hypoxanthine, (0.25 µCi/well) was added and the plates were incubated for 24 h more. The plates were then treated as for *Plasmodium* or MCF7 cells, IC_{50} being determined graphically. The positive control was amphotericine B.

4.4.4. In vitro antimicrobial activity

For antimicrobial activity evaluation, ATCC strains (*S. aureus* ATCC 6538P, *S. epidermidis* ATCC 14990, *E. coli* ATCC 8739, and *C. albicans* ATCC 90028) were used as test microorganisms. Minimal inhibitory concentrations (MIC) were determined by a liquid microdilution method on standard medium in 96-well plates (Trypcase soy broth for bacteria and Sabouraud for *Candida*, (Valentin et al., 1996)). Bactericidal concentration (BC) was determined by subcultures of the MIC plates on square Petri dishes. The positive control was Doxorubicin.

4.5. 4,6,2′-Trihydroxy-6-[10′(Z)-heptadecenyl]-1-cyclohexen-2-one (1a); 1,4,6-trihydroxy-1,2′-epoxy-6-[10′(Z)-heptadecenyl]-2-cyclohexene (1b)

Colourless oil: $[\alpha]_D^{25}$ +22.8° ($CHCl_3$, *c* 2.0); UV (MeOH) λ_{max} ($\log \varepsilon$) nm: 203 (4.00), 225 (3.37); IR ν_{max}^{KBr} 3373 (*br* OH), 3005 (alkenes), 2925, 2854, 1681 (C=O), 1465 (alkanes) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6 and $CDCl_3$) see Tables 2 and 3, ^{13} C NMR (125 MHz, DMSO-d_6 and $CDCl_3$) see Table 1; HR-ESI-MS *m/z*: 381.302 $[M+H]^+$ (calc. for $C_{23}H_{41}O_4$, 381.3005) and 403.283 $[M+Na]^+$ (calc. for $C_{23}H_{40}NaO_4$ 403.2824).

4.6. 1,4,5,2′-Tetrahydroxy-1-[10′(Z)-heptadecenyl]-2-cyclohexene (2)

Colourless oil: $[\alpha]_D^{25}$ +35° ($CHCl_3$, *c* 1.1); UV (MeOH) λ_{max} ($\log \varepsilon$) nm: 203 (3.29); IR ν_{max}^{KBr} 3343 (*br* OH), 3005 (alkenes), 2925, 2854, 1655, 1465 (alkanes) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6 and $CDCl_3$) see Tables 2 and 3, ^{13} C NMR (125 MHz, DMSO-d_6 and $CDCl_3$) see Table 1; HR-ESI-MS *m/z*: 383.313 $[M+H]^+$ (calc. for $C_{23}H_{43}O_4$, 383.3161) and 405.305 $[M+Na]^+$ (calc. for $C_{23}H_{42}NaO_4$ 405.2981).

4.7. 1,3,4,6-Tetrahydroxy-1,2′-epoxy-6-[10′(Z)-heptadecenyl]-cyclohexane (3)

Colourless oil: $[\alpha]_D^{25}$ −9.6° ($CHCl_3$, *c* 1.6); UV (MeOH) λ_{max} ($\log \varepsilon$) nm: 203 (3.14); IR ν_{max}^{KBr} 3524, 3382, 3200 (*br* OH), 2925, 2854, 1731, 1465 (alkanes) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6 and CD_3OD) see Tables 2 and 3, ^{13} C NMR (125 MHz, DMSO-d_6 and CD_3OD) see Table 1; HR-ESI-MS *m/z*: 399.307 $[M+H]^+$ (calc. for $C_{23}H_{43}O_5$, 399.3111) and 421.299 $[M+Na]^+$ (calc. for $C_{23}H_{42}NaO_5$ 421.2930).

Acknowledgements

The authors are grateful to Dr. Véréna Poinsot from Laboratoire des IMRCP (UMR 5623 UPS/CNRS) for high-resolution mass spectra measurements, Séverine Maurel and Edouard Hnawia from UMR 152, for technical assistance in biological evaluations. Pr Joseph Vercauteren (Université Montpellier 1, Faculté des Sciences Pharmaceutiques) is greatly acknowledged for fruitful discussion about NMR results.

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