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New ether diglycosides from *Matayba guianensis* with antiplasmodial activity

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Abstract—Four new ether diglycosides (**1**–**4**), named matayosides A–D, were isolated from the root bark of *Matayba guianensis*, a plant exhibiting in vitro antiplasmodial activity. They were identified as hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-6-*O*-palmitoyl-β-D-glucopyranoside, hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-glucopyranoside, hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-*O*-acetyl-β-D-glucopyranoside and hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-6-*O*-acetyl-β-D-glucopyranoside, respectively. Their structures were established using one- and two-dimensional NMR techniques, mass spectrometry (MS) and MS/MS experiments. The compounds were found to inhibit the growth of *Plasmodium falciparum* in vitro with IC_{50} values ranging from 2.5 to 8.9 μg/ mL.

1. Introduction

Malaria is one of the major infectious diseases that is caused by a protozoan of the genus *Plasmodium*, the most lethal being *Plasmodium falciparum*, which is distributed in many tropical and subtropical regions.[1] The increasing prevalence of malaria exhibiting the resistance of *P. falciparum* to cheap standard treatments, such as chloroquine, has led to the search for new antimalarial compounds.[2]

In our search for natural products with anti-malarial activity, we assayed the anti-*P. falciparum* effects of 204 crude extracts of native plants from Brazilian Cerrado, the country's second most important biome. Some of them were selected for further investigations based on their thresholds of activity as previously defined.[3] Particularly, the hexane extract of the root bark of *Matayba guianensis* Aublet (Sapindaceae) showed a significant in vitro antiplasmodial activity on the chloroquine-resistant strain FcB1 of *P. falciparum* (IC_{50} = 6.1 μg/mL) and a good selectivity towards the parasite when compared to the inhibitory activity of the plant on the mammalian L-6 and MRC-5 cell growth (IC_{50} > 60 μg/mL).

No phytochemical or pharmacological reports have been published in the literature for this species. A previous study on the stem and twigs of *M. arborescens* led to the identification of cleomiscosin A and scopoletin,[4] while the fatty acid composition of the seeds of *M. elaeagnoides* was reported.[5]

The extract of *M. guianensis* was submitted to a series of bioassay-guided fractionations and the most potent antiplasmodial fraction was rechromatographed by combining silica gel column chromatography and MPLC, leading to the isolation of four new ether diglycosides designated as matayosides A–D (**1**–**4**).

Keywords: Antiplasmodial activity; Ether diglycosides; Matayosides A–D; *Matayba guianensis*; Sapindaceae.
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The structures of compounds **1**–**4** were determined on the basis of 1D (^{1}H and ^{13}C) and 2D-NMR techniques (COSY, HSQC, HMBC, NOESY) and by mass spectrometry, including high-resolution ESI-MS, FAB-MS and MS/MS experiments. The monosaccharide composition was determined after acid hydrolysis. The in vitro antiplasmodial activity of purified compounds was evaluated against the chloroquine-resistant strain FcB1 of *P. falciparum*.

2. Results and discussion

The HRFABMS (positive-ion mode) of compound **1** exhibited a pseudo-molecular ion $[M+Na]^+$ at *m*/*z* 937.6232 corresponding to a molecular weight of 914, compatible with a molecular formula of $C_{50}H_{90}O_{14}$. This composition indicated the presence of six unsaturations, of which four were defined by ^{13}C NMR as ester carbonyls (three acetoxy carbonyl groups) and two as cyclic ring systems. The ^{1}H spectrum (Table 1) showed three characteristic acetyl methyl signals at δ 1.97, 2.02, 2.12, a methyl triplet at δ 0.86 (H-16‴, H-16″) and a methyl doublet at δ 1.16, several alkyl protons at δ 1.18–1.28, 10 oxymethine protons between δ 3.25 and δ 5.29 and two oxymethylene protons at δ 4.18 and δ 4.56 suggesting two sugar residues. The presence of sugar moieties was confirmed by the analysis of the HSQC spectrum in which the anomeric protons at δ 4.33 (d, J = 7.7 Hz) and 5.24 (d, J = 1.8 Hz) correlated with carbon signals at δ 101.69 and 97.43. The methyl carbon at δ 17.16, coupling with the doublet methyl proton at δ 1.16 (d, J = 6.2 Hz), indicated the presence of a 6-deoxy sugar unit.

The full assignments of proton and carbon shifts of the sugar moieties in **1** was accomplished by a combination of two-dimensional COSY, HSQC, HMBC and NOESY NMR experiments. The ^{1}H–^{1}H COSY spectrum showed cross-peaks between H-1 and H-2, H-2 and H-3, H-3 and H-4, H-4 and H-5, H-5 and H-6 and also established the spin system H1′–H2′–H3′–H4′–H5′–H6′. The connection of these two partial structures was determined by the long-range ^{1}H–^{13}C correlations deduced from the HMBC spectrum. The anomeric proton H-1′ correlated with C-2, C-3′ and C-5′ while H-4′ showed cross-peak with C-3′, C-5′, C-6′ and 4′-CO. On the other hand, the anomeric proton H-1 correlated with C-1″ and H-4 showed long-range correlations with C-3, C-5 and C-6. Thus, multibond correlations between the anomeric protons revealed two ether linkages C1–O1–C1″ (with an aliphatic chain) and C2–O1′–C1′ for

Table 1. NMR data for matayoside A (**1**) in $CDCl_3$

Position	δC	δH, mult (*J* in Hz)	COSY	HMBC (H to C)	NOESY
1	101.69	4.33, d (7.7)	2	C-1″	3, 5, 1″a, 1″b
2	76.52	3.50, dd (9.1; 7.7)	1, 3	C-1′	4, 1′, 5′
3	77.06	3.64, t (9.1)	2, 4	C-2, C-4	1, 5, 1′
4	70.08	3.25, dd (9.6; 9.1)	3, 5	C-3, C-5, C-6	2
5	73.77	3.35, ddd (9.6; 3.9; 2.3)	4, 6a, 6b		1, 3
6a	62.93	4.18, dd (12.3; 2.3)	5, 6b	C-4, C-5, 6-CO	
6b		4.56, dd (12.3; 3.9)	5, 6a	6-CO	
1′	97.43	5.24, d (1.8)	2′	C-2, C-3′, C-5′	2, 3, 2′
2′	69.67	5.29, dd (3.4; 1.8)	1′, 3′	C-3′, C-4′, 2′-CO	1′
3′	69.27	5.25, dd (10.0; 3.4)	2′, 4′	C-4′, 3′-CO	5′
4′	71.03	5.05, t (10.0)	3′, 5′	C-3′, C-5′, C-6′, 4′-CO	6′
5′	66.37	4.26, dd (10.0; 6.2)	4′, 6′	C-3′, C-4′, C-6′	2, 3′
6′	17.16	1.16, d (6.2)	5′	C-4′, C-5′	4′
2′-CO	170.24				
3′-CO	170.16				
4′-CO	169.88				
2′-CH_3CO	20.88	2.12, s		C-2′, 2′-CO	
3′-CH_3CO	20.70	1.97, s		C-3′, 3′-CO	
4′-CH_3CO	20.72	2.02, s		C-4′, 4′-CO	
1″a	70.35	3.47, dt (9.4; 7.0)	1″b, 2	C-1, C-2″, C-3″	1
1″b		3.87, td (9.4; 7.0)	1″a, 2″	C-1, C-2″, C-3″	1
2″	29.61	1.59, m	1″a, 1″b	C-1″, C-3″	
3″	25.92	1.31, m			
4″–13″	29.69	1.18–1.28, br			
14″	31.88	1.24, br			
15″	22.64	1.27, br		C-14″, C-16″	
16″	14.01	0.86, t (6.8)		C-14″, C-15″	
1‴-CO	175.18				
2‴	34.07	2.36, t (7.9)	3‴	1‴-CO, C-3‴, C-4‴	
3‴	24.82	1.59, m		1‴-CO, C-2‴, C-4‴	
4‴	29.07	1.29, m			
5″–13‴	29.60	1.18–1.28, br			
14‴	31.88	1.24, br			
15‴	22.64	1.27, br			
16‴	14.04	0.86, t (6.8)		C-14‴, C-15‴	

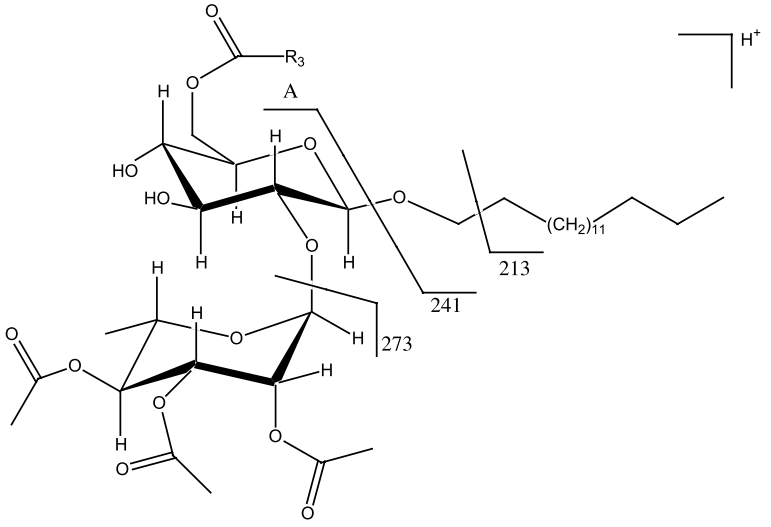
the disaccharide. The fatty acid was found to be attached via an ester linkage to the C-6 oxygen as confirmed the correlations of H-6 and H-2‴ to 1‴-CO. The localisation of the acetyl moieties was determined by the observation of the HMBC correlation between the protons at δ 5.29 (H-2′), 5.25 (H-3′), 5.05 (H-4′) and the acetyl carbonyl carbons at δ 170.24 (2′-CO), 170.16 (3′-CO), 169.88 (4′-CO).

To complete the structure, it was necessary to determine the identities of the disaccharide and the length of the aliphatic chain. The use of NMR did not allow access to this chain.

The compound was analysed by ESI-TOF and FAB-MS, and the results are summarised in Table 3. The first-order ESI-MS showed ion peaks at *m*/*z* 915 and another intense ion at *m*/*z* 932. The MS/MS electrospray ionisation measurements performed on the parent peak at *m*/*z* 915 and at *m*/*z* 932 led to the same fragments at *m*/*z* 673, 213, 153 and an abundant fragment at *m*/*z* 273. In fact, the pseudo-molecular ion at *m*/*z* 937, corresponds to $[M+Na]^+$ and the ion at *m*/*z* 932 to $[M+H_2O]^+$. Formation of $[M+H_2O]^+$ ions could be possibly attributed to the electrospray ionisation mechanism. In a recent study, hydrated ions $[M+H_2O]^+$ produced from sophorolipids were demonstrated to be solvent adducts, and the use of methanol as solvent instead of acetonitrile in the LC/MS experiments suppressed the formation of hydrated ions.[6] The intense fragment peak at *m*/*z* 273, corresponding to $C_{12}H_{17}O_7$, was attributed to the triacetylated 6-deoxy sugar present in compounds **1**–**4** (Fig. 1). The peak at *m*/*z* 673 ($C_{34}H_{57}O_{13}$) can be attributed to the disaccharide fragment (A) formed by the cleavage of the ether linkage between the disaccharide and the alkyl chain. This suggests the presence of a 16-carbon chain for the ester moiety at C-6 of the hexose. The acid responsible for this esterification was therefore identified as palmitic acid or hexadecanoic acid. The expected fragment ion at *m*/*z* 241, according to the molecular weight and corresponding to the ether alkyl chain, was not observed. However, the presence of an ion at *m*/*z* 213 ($C_{15}H_{33}$) could be explained by the loss of CO from the expected fragment ion at *m*/*z* 241 and confirmed the existence of a 16-carbon chain, providing evidence that the etherification was performed with *n*-hexadecanol. The same fragmentation was also observed for compounds **2**–**4**.

Acid hydrolysis of **1** afforded palmitic acid (*m*/*z* 256) and *n*-hexadecanol (*m*/*z* 242), and the monosaccharide components were identified as glucose and rhamnose by co-TLC and MS.[7]

The coupling constant of 7.7 Hz at 4.33 ppm is in agreement with a diaxial coupling between the protons H-1 and H-2 in a β linked D-glucopyranose. However, the value of 1.8 Hz for the other constant excluded a diaxial coupling for H-1′ and H-2′, suggesting an α-glycoside linkage. Thus, the sugar moiety was determined to be 2′,3′,4′-tri-*O*-acetyl neohesperidose (2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose). Accordingly, the structure of this glycolipid (compound **1**) was characterised as hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyrano-



	R_3	A (*m*/*z*)
1	$(CH_2)_{14}CH_3$	673
2	CH_3	519
3	CH_3	519
4	CH_3	477

Figure 1. Observed fragmentations of $[M+H]^+$ pseudo-molecular ion for compounds **1**–**4**.

Table 2. NMR Data for compounds **2**, **3** and **4** in $CDCl_3$

Position	δC	**2** δH, mult (*J* in Hz)	δC	**3** δH, mult (*J* in Hz)	δC	**4** δH, mult (*J* in Hz)
1	101.50	4.33, d (7.7)	101.48	4.39, d (7.7)	101.70	4.32, d (7.7)
2	77.26	3.57, dd (9.1; 7.7)	75.82	3.63, dd (9.4; 7.7)	76.57	3.47, dd (7.7; 9.1)
3	76.33	3.76, t (9.1)	78.49	4.99, t (9.4)	77.14	3.59, t (9.1)
4	71.43	4.78, dd (9.9; 9.1)	74.12	3.47, d ([a])	70.09	3.26, t (9.1)
5	71.65	3.54, ddd (9.9; 5.0; 2.4)	69.72	3.46, d ([a])	73.68	3.34, ddd (9.7; 3.8; 2.2)
6	62.42	4.08, dd (12.2; 2.4)	63.00	4.3, dd (11.9; 1.7)	63.13	4.18, dt (12.3; 2.2)
		4.25, dd (12.2; 5.0)		4.39, dd (11.9; 4.2)		4.51, dd (12.3; 3.8)
3-CO	—		172.52		—	
4-CO	171.02		—		—	
6-CO	170.72		171.70		172.19	
3-CH_3CO	—		20.70	2.15, s	—	
4-CH_3CO	20.80	2.08, s	—		—	
6-CH_3CO	20.77	2.06, s	20.76	2.09, s	20.85	2.11, s
1′	97.65	5.25, d (1.8)	97.56	4.99, d (1.8)	97.47	5.24, d (1.8)
2′	69.69	5.27, dd (3.5; 1.8)	70.22	5.03, dd (3.4; 1.8)	69.70	5.28, dd (1.8; 3.5)
3′	69.26	5.03, dd (10.0; 3.5)	68.66	5.23, dd (10.2; 3.4)	69.28	5.22, dd (3.5; 10.1)
4′	71.11	5.03, t (10.0)	71.22	5.05, t (10.2)	71.07	5.02, t (10.1)
5′	66.50	4.22, dq (10.0; 6.3)	66.53	4.25, dq (10.2; 6.2)	66.40	4.24, dd (6.3; 10.1)
6′	17.24	1.16, d (6.3)	17.18	1.15, d (6.2)	17.20	1.15, d (6.3)
2′-CO	170.18		169.95		170.29	
3′-CO	170.20		169.83		170.23	
4′-CO	169.91		170.08		169.88	
2′-CH_3CO	20.77	1.97, s	20.77	2.12, s	20.89	2.12, s
3′-CH_3CO	20.90	2.11, s	20.58	1.98, s	20.71	1.97, s
4′-CH_3CO	20.77	2.02, s	20.60	2.02, s	20.73	2.02, s
1″	70.42	3.46, td (9.4; 6.8)	70.38	3.5, dt (9.5; 6.9)	70.38	3.44, td (6.9; 9.5)
		3.84, td (9.4; 6.8)		3.85, dt (9.5; 6.9)		3.84, td (6.9; 9.5)
2″	29.57	1.56, m	29.30	1.59, m	29.50	1.59, m
3″	26.04	1.32, br	25.90	1.30, br	25.96	1.31, br
4″–13″	29.69	1.23–1.31, br	29.63	1.22–1.31, br	29.60	1.20–1.31, br
14″	31.92	1.24, br	31.86	1.23, br	31.80	1.23, br
15″	22.68	1.27, br	22.61	1.28, br	22.60	1.27, br
16″	14.09	0.84, t (6.8)	14.03	0.86, t (6.8)	14.05	0.84, t (7.1)

[a] Overlapped, coupling constants were not directly measurable.

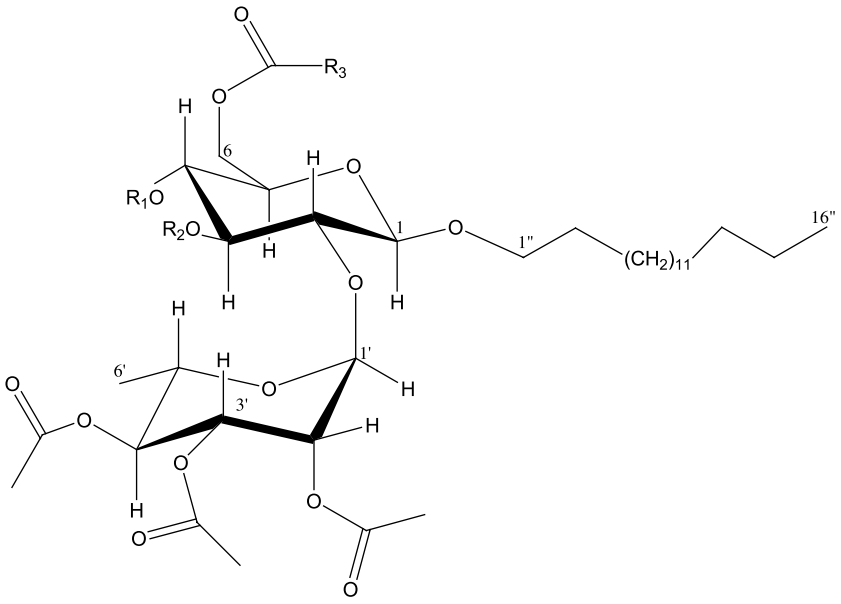
Table 3. Mass spectrometric analysis of matayoside A–D (**1**–**4**)

Compound	$[M+Na]^+$	$[M+H_2O]^+$	$[M+H]^+$	MW	MS–MS (low energy)	FAB-MS
1	937	932	915	914	153, 213, **273**, 673	273, 413, 699, 937
2	783 (–)	778	761	760	153, 213, **273**, 519	273, 413, 519, 783
3	783 (–)	778	761	760	153, 213, **273**, 519	273, 413, 519, 783
4	741	736	719	718	153, 213, **273**, 477	273, 413, 477, 741

syl-(1→2)]-6-*O*-palmitoyl-β-D-glucopyranoside and the trivial name of matayoside A was given.

Compound **2** was obtained as a colourless viscous liquid. The molecular formula was established as $C_{38}H_{64}O_{15}$ by HRFABMS and accounts for seven degrees of unsaturation. The ^{13}C NMR spectrum (Table 2) showed two anomeric methines (δ 97.65 and 101.50), eight oxygen-bearing methines, two oxygen-bearing methylenes, five carbonyl resonances (δ 169.91, 170.18, 170.20, 170.72 and 171.02), four methyls belonging to acetyl groups (δ 20.77–20.90), two alkyl methyl groups (δ 14.09 and 17.24) and numerous overlapping alkyl methylenes. The ^1H NMR spectrum of **2** exhibited signals for five acetyl methyl signals, two sugar anomeric protons, and when compared with that of **1**, H-4 was shifted downfield (+1.53 ppm) whereas H_2-6 were shifted upfield (−0.10 and −0.31 ppm). The HMBC spectrum showed long-range correlations of H-4 with the carbonyl group at δ 171.02, and cross-peaks between the acetyl protons at δ 2.08 with C-4 and 4-CO. Acetyl protons at δ 2.06 correlated with C-6 and 6-CO confirmed the presence of a diacetylated glucose unit at carbons 4 and 6. The ESI-MS-MS of **2** gave fragment peaks at *m*/*z* 273, 213, 519. The presence of the fragment ion at *m*/*z* 519 (A) corresponding to the molecular formula $C_{22}H_{33}O_{14}$ indicated that the disaccharide was pentaacetylated as observed in the NMR spectra, and the alkyl chain was deduced as in **1** to be a 16-carbon chain (Fig. 2).

Acid hydrolysis of **2** followed by TLC analysis with authentic samples of D-glucose and L-rhamnose demonstrated that these monosaccharides were present. The aglycon (*n*-hexadecanol) was identified by MS. Thus, the structure of **2** was elucidated as hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-glucopyranoside, and named matayoside B.



	R_1	R_2	R_3
1	H	H	$(CH_2)_{14}CH_3$
2	Ac	H	CH_3
3	H	Ac	CH_3
4	H	H	CH_3

Figure 2. Ether diglycosides from *M. guianensis*.

Compound **3** exhibited the same molecular formula of $C_{38}H_{64}O_{15}$ as compound **2** based on HRFABMS spectrometry. An identical ESI-MS spectrum was obtained but in the ^{1}H NMR spectrum (Table 2), differences for the signals of H-3 and H-4 were noted. The comparison of the two spectra showed that the proton H-3 was shifted downfield (+1.23) and H-4 was upfield (−1.31) for **3** suggesting that C-4 was deacetylated. These two compounds differ only in the position of the acetyl groups on the disaccharide. In the HMBC spectrum, the cross-peak correlation between H-3 and the carbonyl at δ 172.52 and between H-6 and the acetylated carbonyl carbon at δ 171.70 supported the presence of a diacetylated glucose unit at C-3 and C-6. Therefore, compound **3** was determined to be hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-*O*-acetyl-β-D-glucopyranoside (matayoside C).

The molecular formula of compound **4** was determined as $C_{36}H_{62}O_{14}$ by HRFABMS, which coincided with six degrees of unsaturation. It was also isolated as a viscous liquid. The ^{13}C NMR spectrum (Table 2) of **4** was markedly similar to that of **1** except for the methylene carbons at δ 24.87, 29.09, 34.11. The ^{1}H NMR spectrum of **4** showed an additional acetyl methyl signal at δ 2.11 and the suppression of a triplet at δ 2.36 present in **1**. The 2D NMR experiments on **4** produced very similar results indicating that both **1** and **4** were closely related. Long-range correlations observed for H_2-6 (δ 4.18, 4.51) and CH_3 (δ 2.11) protons with the carbonyl resonating at δ 172.19 supported the presence of an acetylated glucose moiety at C-6 rather than one alkylated by a long chain. It was noticed that this carbonyl was shifted upfield (−2.99) compared to **1**.

The ESI-MS spectrum exhibited pseudo-molecular ions at *m/z* 736 $[M+H_2O]^+$, 719 $[M+H]^+$, 1437 $[2M+H]^+$. The ion fragment observed in the ESI-MS-MS at *m/z* 477 ($C_{20}H_{29}O_{13}$) from the $[M+H]^+$ ion at *m/z* 719 and produced by the loss of the aglycon confirmed that the disaccharide is tetra-acetylated (Fig. 2). Acid hydrolysis of **4** yielded *n*-hexadecanol, D-glucose and L-rhamnose. From this evidence, the structure of **4** was established as hexadecyl-[*O*-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-6-*O*-acetyl-β-D-glucopyranoside, namely matayoside D.

The glycosides isolated from this plant have a common disaccharide unit composed of a tri-acetylated rhamnopyranose and a glucopyranose variously acetylated and attached at C-1 to the aglycon (*n*-hexadecanol). In the case of **1**, an alternative esterification by a fatty acid occurred on the glucose moiety. Few similar ether diglycosides[8–10] have been described in the literature, and related substances, hexadecanol glycosides, have been reported from *Dimocarpus fumatus*, another genus of the Sapindaceae.[11]

Table 4. Antiplasmodial activity of compounds **1**–**4**

Compound	IC_{50} ± SD (μg/mL)	IC_{90} ± SD (μg/mL)
1	7.4 ± 2.0	13.0 ± 3.0
2	3.6 ± 0.7	7.1 ± 1.0
3	8.9 ± 1.1	15.3 ± 1.2
4	2.5 ± 0.3	4.7 ± 0.8

Results are expressed as IC_{50} and IC_{90} values (μg/mL) ± standard deviations. All experiments were realised in triplicate.

Compounds **1**–**4** were evaluated for their in vitro antiplasmodial activity against the chloroquine-resistant strain FcB1 of *P. falciparum*. In comparison to chloroquine, which was used as a positive control, they exhibited IC_{50} values between 2.5 and 8.9 μg/mL, and have to be considered as moderately active (Table 4).

The presence of a fatty acid ester instead of an acetyl group at position C-6 increases the hydrophobicity (**1**) and is unfavourable to the activity compared to the more polar compound **4**. Acetylation at position C-4 does not modify the antiplasmodial activity (**2** and **4**), which decreases in case of esterification at position C-3 (**2** and **3**).

Several constituents isolated from Sapindaceae exhibited haemolytic activity.[12] It was thus important to evaluate the ability of compounds **1**–**4** to lyse human erythrocytes. Indeed, their antiplasmodial activity might be due to their haemolytic properties. No haemolysis of human erythrocytes was observed for compounds **1**, **2** and **3** at the concentration of 100 μg/mL under the same experimental conditions as for the antiplasmodial assay (1% haematocrit, 48 h incubation with the drug). Haemolysis was measured for compound **4** at a concentration of 100 μg/mL (51%, mean of two independent experiments) but no haemolytic activity was observed at a concentration of 25 μg/mL. These results confirmed that the in vitro anti-plasmodial activity of matayoside A–D is not due to haemolysis of human erythrocytes.

It has been demonstrated that amphiphilic compounds can be incorporated into the lipid bilayer of erythrocyte membrane irreversibly and can induce shape transformation of this membrane.[13] In this case, the inhibition of parasite growth observed in vitro could be attributed to indirect effects due to stomatocytic or echinocytic modifications of the host cell membrane.

Non-parasitised erythrocytes were then incubated with increasing concentrations of compounds **1**–**4** under the same conditions as previously described.[13] No lysis of cells and no change of erythrocyte membrane shape in echinocytic forms were observed at concentrations up to 50 μg/mL by phase-contrast microscopy. There was also no evidence of stomatocytic forms. However, because such modification might not be clearly detectable by photonic microscopy, further investigation using transmission electron microscopy will be required to confirm our observations.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin Elmer model 341 polarimeter at 20 °C. IR spectra were taken on a Nicolet Impact 400D spectrophotometer. The UV spectra were recorded on a Kontron spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.61 MHz, respectively, on a Bruker AVANCE-400 spectrometer at 298 K, equipped with ^{1}H-broad-band reverse gradient probehead. Temperature was controlled by a Bruker BCU-05 refrigeration unit and a BVT 3000 control unit. The ^{1}H and ^{13}C NMR chemical shifts are expressed in parts per million relative to TMS, with coupling constants (*J*) given in Hertz. High-resolution mass spectra were recorded on a JEOL MS700 apparatus. Mass spectra data were recorded using an electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in the positive mode (QSTAR Pulsar I of Applied Biosystems). TLC was carried out on precoated Si gel 60 F_{254} plates (Merck). Spots were detected under UV (254 and 366 nm) before spraying with anisaldehyde solution in EtOH followed by heating the plate at 110 °C. Column chromatography was performed on 200–400 mesh silica gel 60 (Merck). Preparative medium-pressure liquid chromatography (MPLC) was performed with a Knauer pump K-120 on Flashsmart cartridges (Si gel 20–40 lm, AIT, France).

3.2. Antiplasmodial activity assay

The chloroquine-resistant FcB1/Colombia strain of *P. falciparum* (IC_{50} value for chloroquine of 0.1 μM) was maintained continuously in vitro on human erythrocytes in RPMI culture medium containing 7.5% of human serum according to Trager and Jensen.[14] The antiplasmodial activity was determined using a modification of the semi-automated microdilution technique.[15] The crude extract was dissolved in dimethylsulfoxide at a concentration of 10 mg/mL and serially diluted with culture medium in 96-well microplates. Asynchronous parasite cultures were added to each well (1% parasitemia and 1% final haematocrit) and the microplates were maintained at 37 °C for 24 h under appropriate atmosphere. [^{3}H]Hypoxanthine (1–5 Ci/mmol; Amersham, Les Ulis, France) was then added to each well (0.5 μCi per well) and the parasites were maintained for further 24 h. The growth inhibition of the parasite by the extract, fractions and isolated products was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture (without drug) maintained on the same plate. Concentrations leading to 50% and 90% inhibition of parasite growth (IC_{50}, IC_{90}) were determined from the dose–response curves. The results were expressed as the mean ± the standard deviations determined from three independent experiments.

3.3. Haemolysis assay

The drug was serially diluted with culture medium (RPMI + 7.5% human serum) in 96-well plates (final

volume 100 μL). Hundred microlitres of normal human erythrocytes in the same medium were added to each well to get finally 1% haematocrit. Plates were incubated 48 h under the same conditions as in the antiplasmodial assay. Plates were then centrifuged and 100 μL of the supernatant was collected. The percentage of red blood cell haemolysis was determined by measuring the absorbance of haemoglobin at 540 nm. Lysis (0%) was defined as the absorbance measured in erythrocyte cultures maintained without drug. Lysis (100%) was defined as the absorbance measured in cultures where the erythrocytes were totally lysed by a freezing and thawing cycle. Final drug concentrations ranged from 100 to 0.1 μg/mL.

3.4. Plant material

M. guianensis Aublet was collected in the Cerrado biome, in the environs of Brasília, Distrito Federal, Brazil, in 2003. This species was identified by Dr. José Elias de Paula, from the Vegetal Anatomy Department of the Biology Institute of the University of Brasília, and the voucher herbarium specimens were deposited in the Herbarium of said University, under the number J. Elias de Paula (UB) 3697.

3.5. Extraction and isolation

Dried and powdered root bark (2.920 kg) of *M. guianensis* was submitted to successive exhaustive extractions with hexane (2×15 L) through a maceration process. The crude extract (11.8 g) was obtained after the evaporation of the solvents under reduced pressure at 40 °C.

This extract (10.365 g) was fractionated on silica gel and eluted with a gradient of cyclohexane–EtOAc to give 25 fractions (F1–F25) on the basis of TLC behaviour. All fractions were tested and two fractions, F24 and F25, showed strong activity with 73% and 97% inhibition at 10 μg/mL, respectively. Chromatography of the most potent fraction (F25, 1.165 g) was carried out over silica gel with a gradient of cyclohexane–EtOAc and yielded 22 subfractions (fractions F25–1 to F25–22). Successive purification of subfraction F25–13 (132 mg) was achieved by MPLC with silica gel on a Flashsmart cartridge (AIT, France) eluted first with 30% EtOAc in cyclohexane, and then with 2% MeOH in CH_2Cl_2 at a flow rate of 5 mL/min, which afforded compounds **1** (5 mg) and **2** (9 mg). Fraction F25–14 (65 mg) was mainly composed of a mixture of **2** and **3**. This mixture was subjected to MPLC with 30% EtOAc in cyclohexane at the flow rate of 5 mL/min. Further separation and purification was achieved on an MPLC column utilizing 20% EtOAc in cyclohexane, yielding 3 mg of compound **3**. Fractions F25–18 to F25–20 contained **4** and were resembled (97 mg) and chromatographed on silica gel using a gradient of 2–5% MeOH in CH_2Cl_2 followed by MPLC column chromatography with the same eluant at the flow rate of 4 mL/min, furnishing compound **4** (6 mg).

3.5.1. Matayoside A (1). Viscous liquid; $[\alpha]_D^{20}$ −22 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.20), 224 (3.76), 281 (3.32) nm; IR ($CHCl_3$) ν_{max} 2937, 2539, 2365, 1644, 1066 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRFABMS *m/z*: 937.6232 $[M+Na]^+$ (calcd for $C_{50}H_{90}O_{14}Na$, 937.6228).

3.5.2. Matayoside B (2). Viscous liquid; $[\alpha]_D^{20}$ −28.8 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.82), 219 (3.39), 264 (2.25) nm; IR ($CHCl_3$) ν_{max} 2918, 2551, 2365, 1657, 1035 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS *m/z*: 783.4147 $[M+Na]^+$ (calcd for $C_{38}H_{64}O_{15}Na$, 783.4143).

3.5.3. Matayoside C (3). Viscous liquid; $[\alpha]_D^{20}$ −14 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (3.51), 221 (3.18), 270 (2.52) nm; IR ($CHCl_3$) ν_{max} 2937, 2533, 2372, 1744, 1370, 1060 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS *m/z*: 783.4135 $[M+Na]^+$ (calcd for $C_{38}H_{64}O_{15}Na$, 783.4143).

3.5.4. Matayoside D (4). Viscous liquid; $[\alpha]_D^{20}$ −36 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.65), 221 (3.26), 269 (3.11) nm; IR ($CHCl_3$) ν_{max} 3391, 2925, 2514, 2365, 1619, 1048 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS *m/z*: 741.4043 $[M+Na]^+$ (calcd for $C_{36}H_{62}O_{14}Na$, 741.4037).

3.5.5. Acid hydrolysis of compounds. Each diglycoside (1 mg) was refluxed in 2 N aqueous CF_3COOH (2 mL) for 3 h.[16] After cooling, the reaction mixture was diluted with 1 mL of H_2O and extracted with CH_2Cl_2. The acidic aqueous layer was co-evaporated with MeOH until neutrality and analysed by TLC with CH_2Cl_2–MeOH–H_2O (8:5:1) and compared with authentic samples. Glucose and rhamnose were identified with R_f 0.27 and 0.44, respectively.

The organic layer was washed with a saturated solution of $NaHCO_3$, dried with $MgSO_4$, filtered, concentrated and then analysed by TLC with CH_2Cl_2–MeOH (98:2).

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