Antiprotozoal Polyacetylenes from the Tanzanian Medicinal Plant *Cussonia zimmermannii*

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From the petroleum ether extract of the root bark of *Cussonia zimmermannii* four polyacetylenes, **1–4**, were isolated, three of which (**1–3**) were active against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Plasmodium falciparum*, and *Leishmania donovani*.

Medicinal plants still are the dominant form of medicine in most countries. More than 75% of the population of our planet is primarily dependent on raw plant products to meet daily health care needs.[1] Most of these plants and their constituents still have not been identified.

In Tanzania, for example, only a small number of the plants used by traditional healers have been identified. Haerdi described 625 plants used by healers in villages around the town of Ifakara in central Tanzania.[2] Kokwaro listed over 1000 East African medicinal plants,[3] which are used by traditional healers in Kenya and/or Tanzania. Hedberg et al. listed 153 plants, which are used in the northeastern part of Tanzania.[4] Chhabra et al.[5,6] mentioned 146 plants used by healers in five regions of eastern Tanzania: Coast, Dar Es Salaam, Kilimanjaro, Morogoro, and Tanga. Some Tanzanian medicinal plants have been screened for antiprotozoal activity, and active plant extracts have been investigated phytochemically.[7–11]

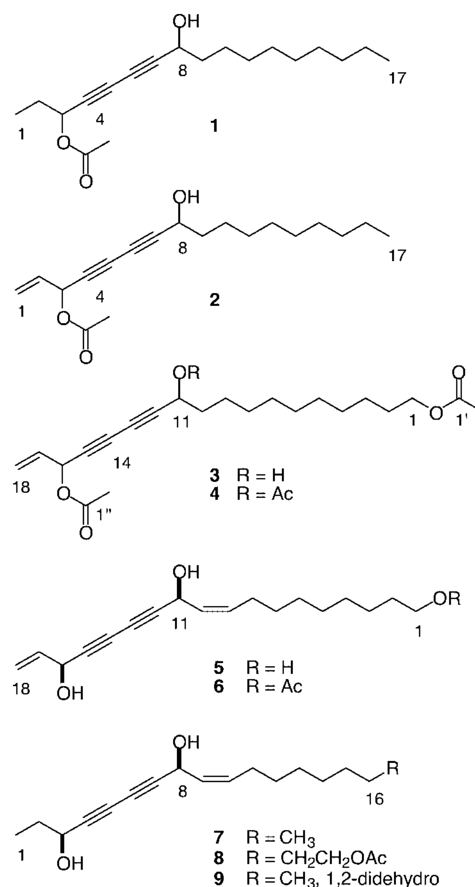
Several diseases, which are of worldwide importance, including amoebiasis, giardiasis, leishmaniasis, malaria, and trypanosomiasis, are caused by protozoa.[12] The drugs used against these diseases are old, show adverse effects, and lose their efficacy. Therefore, there is a great need for the development of new antiprotozoal drugs with novel structures and novel modes of action.

African trypanosomiasis, also known as sleeping sickness, is a neglected disease that is caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*.[13,14] The disease was largely controlled in the 1960s, but it re-emerged in the 1980s and today 60 million people are exposed to it. Forty-eight thousand cases were reported in 2004,[15] but the disease is underreported. The drugs currently used for the treatment of sleeping sickness have major drawbacks in efficacy, safety, and compliance.[16]

Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the poor countries of the world.[17] Four parasite species of the genus *Plasmodium* infect human beings, but nearly all malaria deaths are caused by *Plasmodium falciparum*.[18,19] Currently, more than 10 drugs are available for the treatment of malaria,[20] but drug resistance and affordability are a problem.

Leishmaniasis is a complex of diseases caused by the trypanosomatid parasites *Leishmania* spp. The disease is endemic to many densely populated regions of the world.[21] The drugs available for its treatment are either old and toxic (pentavalent antimonials) or more recently developed but not affordable for endemic countries.[22]

We report here the isolation of four polyacetylenes, **1–4** (called MS-1, MS-3, MS-4, and MS-5, respectively, in our earlier paper[23]), from the petroleum ether extract of the root bark of *Cussonia zimmermannii* Harms. Compounds **1–3** were subjected to biological tests and proved to be active against the agents causing sleeping sickness, malaria, or leishmaniasis. In addition, these compounds proved to be potent and selective modulators of the $GABA_A$ receptor.[23]



Results and Discussion

Selection of the Plants. Material from 13 Tanzanian medicinal plants was collected. The plant species were selected on the basis of the information obtained from the previous work by Gessler[9] and by Freiburghaus.[10] The plant material was dried, hackled, powdered, and then sequentially extracted with petroleum ether, CH_2Cl_2, MeOH, and H_2O. The crude extracts thus obtained were screened for biological activity.

Among the 140 crude extracts tested, 51 showed significant activity either in the antitrypanosomal and/or in the antiplasmodial assay (IC_{50} <5 μg/mL). They were further tested for cytotoxicity. Following these tests, the petroleum ether extract of the root bark of *C. zimmermannii* proved to be the most promising (antitrypa-

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nosomal activity: $IC_{50} = 4.8$ μg/mL, antiplasmodial activity: $IC_{50} = 3.3$ μg/mL). It was selected for fractionation in view of the isolation of single active constituents.

C. zimmermannii (Araliaceae) is a tree growing up to 25 m. Local names are msopole (Kihehe, Kisagara), mtumbitumbi (Kingindo), and mutolondo. The plant occurs in Kenya (Kwale and Tana River Districts), and Tanzania (Tanga, Uzaramo, and Lindi Districts). It grows in lowland rain forest, lowland dry evergreen forest, and woodlands of 0–400 m.[24]

In traditional medicine a decoction of the root of *C. zimmermannii* is used to treat malaria. The marrow of the stem and branches is taken orally against epilepsy, and a decoction of the root serves to relieve labor pain.[2] An infusion of the leaves is used as a wash for people suffering from fever or the ague. A decoction of the roots is taken as a remedy for gonorrhoea.[3] Roots of *C. zimmermannii* along with roots of *Deinbollia borbonica* Scheff. f. are cooked with chicken and the resulting soup is ingested for hypertensive encephalopathy, post-partum hemorrhage, and mental illness.[5]

Of the genus *Cussonia*, 51 constituents from 10 different species have been described hitherto in the literature. Most of them belong to the classes of triterpenoid saponins and diterpenoid glycosides. There is only one species, *C. barteri*,[25] that is known to contain polyacetylenes; the compounds isolated are (9*Z*,11*S*,16*S*)- octadeca-9,17-diene-12,14-diyne-1,11,16-triol (**5**) and the corresponding acetate **6**.

Isolation and Structure Determination. The petroleum ether extract from 717 g of *Cussonia zimmermannii* Harms root bark was subjected to bioassay-guided fractionation using preparative scale MPLC and HPLC techniques. In this way, the polyacetylenes **1** (36.5 mg), **2** (81.2 mg), **3** (221.7 mg), **4** (1.4 mg), and stigmasterol (17.3 mg) were obtained.

Compound **1** was a yellowish, viscous oil with a negative specific rotation ($[\alpha]_D = -94.5$). High-resolution mass spectrometry yielded a mass of 306.21971 for the molecular ion, pointing to the molecular formula $C_{19}H_{30}O_3$. The IR absorption bands indicated the presence of hydroxy groups (3424 cm^{-1}), saturated hydrocarbon moieties (2926, 2855, 1464, and 1372 cm^{-1}), C–C triple bonds (2253 and 2156 cm^{-1}), and esters/acetates (1746 and 1231 cm^{-1}). The ^{13}C NMR spectrum (see Table 1) showed 19 resonances, which corresponded to the molecular formula derived from HREIMS. The DEPT135 spectrum showed 14 resonances for protonated carbons, five methyls or methines, and nine methylenes. Accordingly, the molecule contained five quaternary carbon atoms, which was confirmed by the HMQC measurement. The quaternary carbon atom at δ_C 169.9 was attributed to an ester carbonyl group, and the other four had to be connected by conjugated triple bonds. The suggestion of a diyne structure was in agreement with the acetylene bands in the IR spectrum, the UV absorptions,[26] and comparison with literature data.[25]

Information from the HMQC cross-peaks and the corresponding ^1H NMR integrals indicated two methines and three methyls. Therefore the molecule must contain the following structural components: one COO, four quaternary C atoms (conjugated triple bonds), two CH, nine CH_2, three CH_3, and one OH.

The ^1H NMR spectrum showed two triplets for oxymethines, three methyl resonances (two triplets and a singlet), a triplet for an OH, three methylenes (two multiplets and a broad quintet), and a broad multiplet at δ_H 1.31–1.26 corresponding to 12 protons reminiscent of an aliphatic chain. A ^1H–^1H COSY experiment revealed two separate spin systems. The first showed a methyl at δ 1.02 (3 H-1) that was coupled to a methylene at δ 1.80 (2 H-2), which was further coupled to the oxygen-bearing methine at δ 5.35 (H-3) marking the end of this spin system. The second spin system started at the second oxygen-bearing methine at δ 4.42 (H-8), which was coupled to the OH proton at δ 1.75 and the methylene at δ 1.70 (2 H-9), which was coupled to a further methylene at δ 1.43 (2 H-10). Beyond this, the spin system extended to the broad methylene multiplet at δ 1.31–1.26 (m) and could not be traced further with certainty.

The ^{13}C NMR resonances were correlated with the corresponding ^1H resonances by an HMQC experiment, and the structural fragments were linked using long-range heteronuclear correlations (HMBC experiment, Figure 1 and Table 1). Key correlations were that of the carbonyl resonance at δ_C 169.9 (C-1′) with the methyl proton resonance at δ_H 2.09 (3 H-1′) over two bonds, indicating an acetate. The three-bond correlation observed between the carbonyl carbon resonance at δ_C 169.9 (C-1′) and the methine proton at δ_H 5.35 (H-3) indicated that the acetoxy group was located at C-3. The acetylene carbon resonance at δ_C 80.6 (C-7) showed a two-bond correlation with the proton of the hydroxy-bearing methine at δ_H 4.42 (H-8) and a three-bond correlation with the methylene at δ_H 1.70 (2 H-9). On the opposite side of the diyne, correlations of the acetylene carbon at δ_C 76.7 (C-4) over two bonds with the methine proton at δ_H 5.35 (H-3) and over three bonds with the methylene at δ_H 1.80 (2 H-2) were observed. The diyne chain was therefore situated between the acetoxy bearing C-3 and the hydroxy bearing C-8. The carbon resonance at δ_C 69.3 (C-5) was assigned from the strong three-bond correlation observed with the methine proton at δ_H 5.35 (H-3) and the weak four-bond correlation with the methine proton at δ_H 4.42 (H-8). The resonance of the second inner alkyne carbon at δ_C 68.8 (C-6) correlated strongly over three bonds with the methine proton at δ_H 4.42 (H-8) and weakly over four bonds with the methine proton at δ_H 5.35 (H-3). The additional cross-peaks observed (see Table 1 and Figure 1) corroborated the assignments and structural connections made from the COSY and HMQC experiments.

On the basis of the above data MS-1 (**1**) was identified as 8-hydroxyheptadeca-4,6-diyn-3-yl acetate. The comparison of the spectroscopic data with those of closely related structures, such as **5**, **6**,[25] **7**, and **8**,[27] showed good agreement.

In a similar way, the other three polyacetylenes were identified from their spectroscopic data as 8-hydroxyheptadeca-1-ene-4,6-diyn-3-yl acetate (**2**), 16-acetoxy-11-hydroxyoctadeca-17-ene-12,14-diynyl acetate (**3**), and 11,16-diacetoxyoctadeca-17-ene-12,14-diynyl acetate (**4**), respectively. Stigmasterol was identified by comparison with an authentic sample.

Absolute or relative configurations could not be determined due to the fact that for none of the compounds isolated could a crystalline derivative be obtained that was suitable for an X-ray structure determination. Lack of material after the bioassaying was done precluded chemical means of defining absolute configuration at, for example, C-8 in compound **1**.

Besides compounds **5** and **6**, several other polyacetylenes have been isolated from members of the Araliacea family. The compounds we isolated from *C. zimmermannii* are structurally most closely related to falcarindiol (**9**), lacking, however, the 9,10-double bond of the latter. Falcarindiol had been isolated from *Schefflera digitata* and was shown to have an inhibitory effect on 5-lipoxygenase.[28]

Biological Activity. The results of the antiparasitic (*T. b. rhodesiense, P. falciparum, T. cruzi, and L. donovani* axenic and in infected macrophages) and the cytotoxicity testing of the isolated pure compounds **1**–**3** of *C. zimmermannii* and the selectivity indices of the compounds **2** and **3** are presented in Table 2. The standard drugs melarsoprol, chloroquine, artemisinin, benznidazole, miltefosine, and mefloquine were included for comparison. Compound **4** was not tested, due to the small amount of material isolated.

Of the three polyacetylenes tested, **1** proved to be the least active compound in all antiparasitic *in vitro* assays. Compound **2** showed promising activities in the *T. cruzi* and *L. donovani* (axenic and in infected macrophages) assays with IC_{50} values of 0.65, 0.13, and 0.32 μM, respectively (0.20, 0.039, and 0.098 μg/mL). The respective IC_{50} values of the standard drugs were 2.4, 0.44, and 0.71 μM (0.62, 0.18, and 0.29 μg/mL). The cytotoxicity was relatively high; therefore, the selectivity with SI values of 18 and

Table 1. NMR Spectroscopic Data for Compounds **1**–**4**

	1					**2**				
position	δ_C, mult.	δ_H, (J in Hz)	COSY	HMQC	HMBC[a]	δ_C, mult.	δ_H, (J in Hz)	COSY	HMQC	HMBC[a]
1	9.3, CH$_3$	1.02, t (7.4)		+++	2, 3	119.8, CH$_2$	5.55, d (16.8) 5.35, d (10.0)		++ ++	2, 3
2	27.8, CH$_2$	1.80, m	1 (+++)	+++	1, 3, 4	131.9, CH	5.86, m	1 (+++)	+	4, 3
3	65.3, CH	5.35, t (6.5)	2 (+++)	++	1, 2, 4, 5, 6, 1′	64.5, CH	5.91, m	2 (+++)	+++	1, 2, 4, 5, 6, 1′
4	76.7, C					74.4, C				
5	69.3, C					70.8, C				
6	68.8, C					68.6, C				
7	80.6, C					81.3, C				
8	62.9, CH	4.42, t (6.6)	9 (++), OH (+++)	++	5, 6, 7, 9, 10	62.9, CH	4.43 br, t (6.5)	9 (+++), OH (+)	+++	
9	37.5, CH$_2$	1.70, m	10 (+++)	+++	7, 8, 10	37.4, CH$_2$	1.70, m	10 (+++)	+++	7, 8, 10
10	25.0, CH$_2$	1.43 br, quint. (7.3)	11 (+++)	+++	11–14	25.0, CH$_2$	1.43 br, quint. (7.5)	11 (+++)	+++	8, 9, 11–14
11	29.51[b], CH$_2$					29.51[b], CH$_2$				
12	29.48[b], CH$_2$					29.48[b], CH$_2$				
13	29.3[b], CH$_2$	1.31–1.26, m	17 (+++)	+++	2, 10, 11–14, 15, 16	29.3[b], CH$_2$	1.31–1.22, m	17 (+++)	+++	10, 11–14, 15, 16
14	29.2[b], CH$_2$					29.2[b], CH$_2$				
15	31.9, CH$_2$					31.9, CH$_2$				
16	22.7, CH$_2$					22.7, CH$_2$				
17	14.1, CH$_3$	0.88, t (7.0)		+++	15, 16	14.1, CH$_3$	0.88, t (7.0)		+++	15, 16
OH		1.75, d (5.8)					1.81 br, s			
1′	169.9, C					169.5, C				
2′	20.9, CH$_3$	2.09, s		+++	1′	20.9, CH$_3$	2.11, s		+++	1′

	3					**4**				
position	δ_C, mult.	δ_H, (J in Hz)	COSY	HMQC	HMBC[a]	δ_C, mult.	δ_H, (J in Hz)	COSY	HMQC	HMBC[a]
18	119.7, CH$_2$	5.54, d (16.8) 5.34, d (10.0)		+++ +++	16, 17	119.8, CH$_2$	5.54, d (16.8) 5.35, d (9.7)		+ +	
17	131.9, CH	5.85, m	18 (++)	++	15, 16	131.8, CH	5.85, m	18 (++)		16
16	64.5, CH	5.90, m	17 (++)	+++	13, 14, 15, 17, 18, 1″	64.4, CH	5.90, m	17 (++)	+	17, 1″
15	74.3, C					74.5, C				
14	70.9, C					70.7, C				
13	68.5, C					69.0, C				
12	81.4, C					77.7, C				
11	62.8, CH	4.42 br, t (6.3)	10 (+++), OH (+)	+++	9, 10, 12, 13, 14	64.1, CH	5.37, t (7.4)	10 (+++)	++	10, 12, 13, 1‴
10	37.4, CH$_2$	1.70, m	9 (++)	+++	4–8, 9, 11, 12	34.4, CH$_2$	1.76, m	9 (+++)	+	4–8, 11, 12
9	25.0, CH$_2$	1.43 br, quint. (7.2)	8 (+++)	+++	4–8, 10, 11	24.9, CH$_2$	1.42, m	8 (+++)	+	
8	29.44[b], CH$_2$					29.5[b], CH$_2$				
7	29.41[b], CH$_2$					29.42[b], CH$_2$				
6	29.37[b], CH$_2$	1.34–1.24, m		+++	1, 2, 3, 4–8, 9	29.37[b], CH$_2$	1.33–1.23, m		+	2, 4–8
5	29.21[b], CH$_2$					29.2[b], CH$_2$				
4	29.17[b], CH$_2$					29.1[b], CH$_2$				
3	25.9, CH$_2$					25.9, CH$_2$				
2	28.6, CH$_2$	1.61, quint. (7.2)	3 (+++)	+++	1, 3, 4–8	28.6, CH$_2$	1.61, m	3 (+++)	+	1, 3, 4–8
1	64.7, CH$_2$	4.04, t (6.8)	2 (+++)	+++	2, 3, 4–8, 1′	64.7, CH$_2$	4.05, t (6.8)	2 (+++)	++	2, 3, 1′
OH		2.02 br, s								
1″	169.5, C					169.5, C				
2″	20.9, CH$_3$	2.10, s		+++	1″	20.90[b], CH$_3$	2.10, s		+++	1″
1′	171.3, C					171.3, C				
2′	21.0, CH$_3$	2.04, s		+++	1′	21.1, CH$_3$	2.05, s		+++	1′
1‴						169.8, C				
2‴						20.88[b], CH$_3$	2.08, s		+++	1‴

[a] HMBC correlations, optimized for 7 Hz, are from proton(s) stated to the indicated carbon(s). [b] Similar assignments within a column may be interchanged.

37, respectively (*T. cruzi* and *L. donovani* in infected macrophages), was in a moderate range. Polyacetylene **3** also showed interesting activities in the *T. cruzi* and *L. donovani* (axenic) assays with IC$_{50}$ values of 0.40 and 0.14 μM, respectively (0.15 and 0.054 μg/mL). The SI value of 150 (*T. cruzi*) was high. Compared with the standard drugs, the activities of **2** and **3** against *T. b. rhodesiense* and *P. falciparum* were in a moderate range. The antileishmanial activity of **2** and **3** is remarkable, especially the activity of **2** in the macrophage assay.

Experimental Section

General Experimental Procedures. Solvents used: MPLC and recrystallization: technical grade, distilled; extraction: distilled twice; HPLC: HPLC-quality. Solvent evaporation was performed on a Büchi Rotavapor RE 111 with a H_2O bath at 40 °C.

Optical rotations were measured on a Perkin Elmer polarimeter 341 in a 10 cm cuvette at 20 °C. UV/vis spectra were obtained from a Perkin-Elmer Lamda Bio 40 UV/vis spectrometer. IR spectra were measured on a Perkin-Elmer model 1600 FT-IR spectrophotometer.

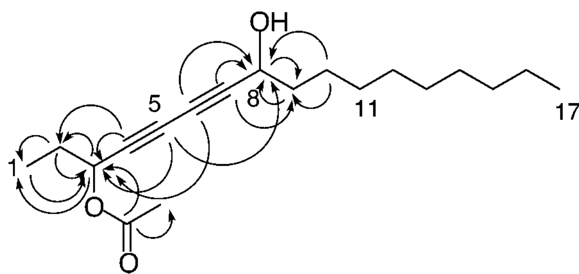

Figure 1. C→H correlations obtained from the HMBC experiment of **1** in $CDCl_3$ (optimized for $^nJ_{CH}$ = 7 Hz (n = 2 or 3)).

Resonance frequencies are given in cm^{-1}; intensities: s = strong, m = medium, and w = weak.

NMR spectra were measured in $CDCl_3$ on a Bruker VRX 500 (^1H NMR 500 MHz; ^{13}C NMR 125 MHz) at our Department with the assistance of Dr. K. Kulicke. The chemical shifts are given in ppm relative to internal tetramethylsilane (δ = 0). ^{13}C NMR spectra were measured using broad-band decoupling with $CDCl_3$ as the reference (δ = 77.0). Multiplicities were derived from DEPT experiments. Cross-peak intensities in COSY and HMQC experiments: +: weak, ++: medium, and +++: strong.

Mass spectra were measured by Dr. H. Nadig at the Department of Chemistry of the University of Basel on a VG 70S (EI) spectrometer and a Finnigan MAT 312 (FAB). *m/z* values are reported with the corresponding relative intensities in parentheses.

MPLC was performed with a Büchi B-680 system, consisting of a Büchi B-688 chromatography pump, a Büchi B-687 gradient former, a Knauer variable-wavelength monitor set to 254 nm, a Knauer strip chart recorder, and a Büchi B-684 fraction collector.

Analytical HPLC was performed with an HP 1090 system from Hewlett Packard. The data were processed on a HP Chemstation from Hewlett Packard.

Preparative HPLC was performed with a Waters 600 system consisting of a Waters 600 multisolvent delivery system, a Waters 996 photodiode array detector, a Waters 600 controller, and a Waters fraction collector. The spectra were processed with the software Millennium V3.1. from Waters.

Stigmasterol (authentic sample for comparison) was bought from Acros Organics. Chemicals and materials used in the antiparasitic and cytotoxicity testings were standard materials used in the routine work of the laboratories of the Swiss Tropical Institute, Basel.

Plant Material. Plant samples were collected at the end of the wet season in June and July 2001 from Pugu Forest (50 km west of Dar Es Salaam) and Chalinze region (100 km west of Dar Es Saalam) in Tanzania. The plants were identified botanically by Mr. L. B. Mwasumbi of the Institute of Botany of the University of Dar Es Salaam. *C. zimmermannii* was found to be identical to the voucher specimen (no. 12240) deposited at the Herbarium of the University of Dar Es Salaam, Tanzania. Hackling of dried plant material was performed with a Fuchs mill type MM 125 H. Powdering of hackled plant material was performed with a Miostar coffee grinder.

Crude Extracts for Preliminary *in Vitro* Testing. Samples of about 35 g of the ground plant material were consecutively extracted with two portions (350 mL) each of petroleum ether (40–60 °C), CH_2Cl_2, MeOH, and H_2O at room temperature under stirring for 24 h per portion. After each extraction step the mixture was left to stand and then filtered. Petroleum ether and CH_2Cl_2 mixtures were filtered under normal pressure, and MeOH and H_2O mixtures were filtered under vacuum. Same solvent filtrates were combined and concentrated at 40 °C. Lipophilic filtrates were dried under high vacuum; aqueous filtrates were freeze-dried. The extracts obtained were weighed and then stored at 4 °C.

Isolation of Constituents from the Petroleum Ether Extract of the Root Bark of *C. zimmermannii*. Ground root bark (717 g) was extracted by stirring with three portions (3.5 l each) of petroleum ether (40–60 °C) at room temperature for 24 h per portion. After each aliquot the mixture was left to stand and then filtered under normal pressure. The filtrates were combined, evaporated at 40 °C, and dried under high vacuum.

The crude plant extract (4.7 g) was then subjected to MPLC followed by preparative scale HPLC as described below. Fractions obtained with organic solvents were evaporated at 40 °C and then dried under high vacuum. From fractions obtained with organic solvent–H_2O mixtures the organic solvent was removed by evaporation at 40 °C, and the remaining aqueous solution was then freeze dried. After each fractionation step the fractions were checked for purity by analytical HPLC (Knauer Eurospher 100 C18, 5 μm, 250 × 4.6 mm i.d.; gradient of MeCN with 90–10% H_2O).

The crude residue from the petroleum ether extract was first fractionated by MPLC (Merck LiChroprep Si 60, 15–25 μm, 460 × 36 mm i.d.; gradient of *n*-hexane with 0–30% EtOAc). Peaks no. 3 and 4 were further fractionated.

The material from peak no. 3 (1.00 g) was rechromatographed (MPLC; Merck LiChroprep Si 60, 15–25 μm, 460 × 26 mm i.d.; gradient of *n*-hexane with 0–30% EtOAc). Peaks no. 6 and 7 from this run were treated further. The material from peak no. 6 (310 mg) was fractionated using MPLC (Eurosil Bioselect 100–20 Diol 20 μm, 460 × 15 mm i.d.; gradient of *n*-hexane with 2–10% EtOAc). Rechromatography of the main peak (277 mg) with preparative HPLC (Knauer Eurospher 100 C18, 7 μm, 250 × 16.0 mm i.d.; gradient of MeCN with 40–20% H_2O) gave crude **2** (95.8 mg) and crude **1** (47.6 mg). Purification by preparative HPLC (Knauer Eurospher 100 C18, 7 μm, 250 × 16.0 mm i.d.; gradients of MeCN with 35–10 or 30–10% H_2O) yielded pure **2** (76.4 mg) and **1** (36.5 mg), respectively. The material from peak no. 7 (102 mg) was subjected to MPLC (Merck LiChroprep Si 60, 15–25 μm, 460 × 15 mm i.d.; gradient of *n*-hexane with 5–50% EtOAc). The material from the main peak (60.0 mg) of this run was precipitated in fractions from *n*-hexane, giving crude stigmasterol (29.1 mg) and crude **4** (28.4 mg). This was subjected to preparative HPLC (Knauer Eurospher 100 C18, 5 μm, 250 × 16.0 mm i.d.; gradient of MeCN with 45–10% H_2O), giving pure **4** (1.4 mg).

The material from peak no. 4 of the initial MPLC run (940 mg) was rechromatographed (MPLC; Merck LiChroprep Si 60, 15–25 μm, 460 × 26 mm i.d.; gradient of *n*-hexane with 0–50% EtOAc). The material from peak no. 7 (551 mg) was subjected to HPLC (Knauer Eurospher 100 C18, 5 μm, 250 × 16.0 mm i.d.; gradient of MeCN with 45–35% H_2O) to give crude **3** (267.3 mg), which was then purified (HPLC; Knauer LiChrospher 100 DIOL, 10 μm, 250 × 16.0 mm i.d.; *n*-hexane–CH_2Cl_2, 1:1), yielding pure **3** (222 mg).

8-Hydroxyheptadeca-4,6-diyn-3-yl ethanoate (1): yellowish, viscous oil; $[\alpha]^{20}_D$ −94.5 (c 0.78, $CHCl_3$/0.75% ethanol); UV ($CHCl_3$/0.75% ethanol) λ_{max} (ε) 201.9 (282), 207.0 (308), 209.0 (350), 219.8 (395), 223.9 (440), 244.7 (848), 258.3 (589), 281.8 (196) nm; IR (NaCl) ν_{max} 3424m, 2926s, 2855s, 2253w, 2156w, 1746s, 1464m, 1372m, 1231s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; COSY (500 MHz, $CDCl_3$) see Table 1; HMQC (125 MHz, $CDCl_3$) see Table 1; HMBC (125 MHz, $CDCl_3$) see Table 1; EIMS (70 eV, ca. 200 °C) *m/z* 306 $[M]^+$ (84), 291 (20),

Table 2. Antiparasitic and Cytotoxic Activity of **1**, **2**, **3**, and Some Standard Drugs[a]

compound	IC_{50} *T. brucei Rhodesiense* [μM] ([μg/mL])	IC_{50} *P. falciparum* [μM] ([μg/mL])	IC_{50} *T. cruzi* [μM] ([μg/mL])	IC_{50} *L. donovani* axenic [μM] ([μg/mL])	IC_{50} *L. donovani in* infected macrophages [μM] ([μg/mL])	IC_{50} cytotoxicity (L-6 cells) [μM] ([μg/mL])	SI[b] *T. cruzi*	SI[b] *L. donovani in* infected macrophages
1	18 (5.4)	19 (5.9)	26 (7.9)	7.8 (2.4)	>10 (>3)	54 (17)	2.1	<5.5
2	0.46 (0.14)	1.4 (0.44)	0.65 (0.20)	0.13 (0.039)	0.32 (0.098)	12 (3.6)	18	37
3	1.1 (0.42)	2.2 (0.84)	0.40 (0.15)	0.14 (0.054)	2.3 (0.85)	58 (22)	150	26
standard drugs	0.0058 (0.0023)[c]	0.084 (0.027)[d] 0.0043 (0.0012)[e]	2.4 (0.62)[f]	0.44 (0.18)[g]	0.71 (0.29)[g]	0.019 (0.0080)[h]		

[a] All samples were tested in duplicate (n = 2) in at least two independent assays. [b] Selectivity index = IC_{50} for L-6 cells /IC_{50} for parasite. [c] Melarsoprol. [d] Chloroquine. [e] Artemisinin. [f] Benznidazole. [g] Miltefosine. [h] Podophyllotoxin.

264 (6), 249 (26), 217 (10), 175 (17), 161 (32), 133 (33), 119 (50), 91 (68), 57 (33), 55 (36), 43 (100); HREIMS *m/z* 306.21971 (calcd for $C_{19}H_{30}O_3$, 306.21950).

8-Hydroxyheptadeca-1-ene-4,6-diyn-3-yl ethanoate (2): yellowish, viscous oil; $[\alpha]^{20}_D$ −28.0 (*c* 0.85, $CHCl_3$/0.75% ethanol); UV ($CHCl_3$/0.75% ethanol) λ_{max} (ε) 201.1 (369), 204.0 (351), 208.0 (495), 209.9 (455), 214.0 (567), 216.0 (548), 219.1 (527), 222.1 (642), 245.9 (1245), 259.6 (955), 283.1 (456) nm; IR (NaCl) ν_{max} 3426m, 3088w, 2926s, 2856s, 2255w, 2157w, 1746s, 1650w, 1461m, 1372m, 1225s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; COSY (500 MHz, $CDCl_3$) see Table 1; HMQC (125 MHz, $CDCl_3$) see Table 1; HMBC (125 MHz, $CDCl_3$) see Table 1; FABMS (NBA + KCl) *m/z* 343 $[M + K]^+$ (36), 287 (12), 245 (12), 227 (8), 137 (17), 91 (33), 43 (100); EIMS *m/z* 303 $[M - 1]^+$ (20), 201 (14), 178 (78), 159 (44), 135 (47), 107 (65), 79 (38), 43 (100); HREIMS *m/z* 303.19530 (calcd for $C_{19}H_{27}O_3$, 303.19602).

16-Acetoxy-11-hydroxyoctadeca-17-ene-12,14-diynyl ethanoate (3): yellowish, viscous oil; $[\alpha]^{20}_D$ −26.4 (*c* 0.81, $CHCl_3$/0.75% ethanol); UV ($CHCl_3$/0.75% ethanol) λ_{max} (ε) 202.0 (254), 207.0 (302), 209.1 (353), 212.9 (352), 245.8 (984), 259.7 (601) nm; IR (NaCl) ν_{max} 3444m, 3086w, 3031w, 2922s, 2844s, 2256w, 2156w, 1739s, 1644w, 1467w, 1367w, 1233s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; COSY (500 MHz, $CDCl_3$) see Table 1; HMQC (125 MHz, $CDCl_3$) see Table 1; HMBC (125 MHz, $CDCl_3$) see Table 1; FABMS (NBA + KCl) *m/z* 415 $[M + K]^+$ (32), 377 (2), 359 (5), 317 (11), 299 (12), 136 (13), 91 (24), 55 (35), 43 (100); HREIMS *m/z* 376.22369 (calcd for $C_{22}H_{32}O_5$, 376.22497).

11,16-Diacetoxyoctadeca-17-ene-12,14-diynyl ethanoate (4): yellowish, viscous oil; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; COSY (500 MHz, $CDCl_3$) see Table 1; HMQC (125 MHz, $CDCl_3$) see Table 1; HMBC (125 MHz, $CDCl_3$) see Table 1; FABMS (NBA + KCl) *m/z* 457 $[M + K]^+$ (94), 419 $[M + 1]^+$ (8), 359 (99), 317 (19), 171 (9), 115 (15), 55 (35), 43 (100).

Antiparasitic and Cytotoxicity Tests. The antiparasitic and the cytotoxicity assays were performed according to Standard Operation Procedures (SOP) of the Swiss Tropical Institute, Basel. Sample stock solutions of 10 mg/mL were prepared by dissolving the weighed samples in DMSO. The solutions were kept at room temperature until use. Each assay was performed in duplicate ($n = 2$) and repeated at least once. For the performance of the antiparasitic and cytotoxicity assays Costar 96-well microtiter plates were used.

The assays for *T. b. rhodesiense* and *T. cruzi* were based on the LILIT (long incubation, low inoculation test)[29] with minor modifications. For *T. b. rhodesiense* the Alamar Blue assay with fluorometric reading was used,[30] and the concentration at which the parasite growth was inhibited by 50% (IC_{50}) was calculated by linear interpolation between the two adjacent drug concentrations above and below the 50% incorporation line.[31] *T. cruzi* assays were performed using reporter gene transfected parasites and photometric evaluation.[32] Antiplasmodial activity was assayed as previously described by *Desjardins* et al.[33] The procedure uses the uptake of $[^3H]$hypoxanthine by parasites as an indicator for viability. Antileishmanial activity (*L. donovani*) was determined using axenic amastigote forms[32] and also leishmania-infected mouse macrophages.[34] Cytotoxicity assays were performed using the Alamar Blue assay[30] and L-6 cells (rat skeletal myoblasts).

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