Limonoid Orthoacetates and Antiprotozoal Compounds from the Roots of *Pseudocedrela kotschyi*

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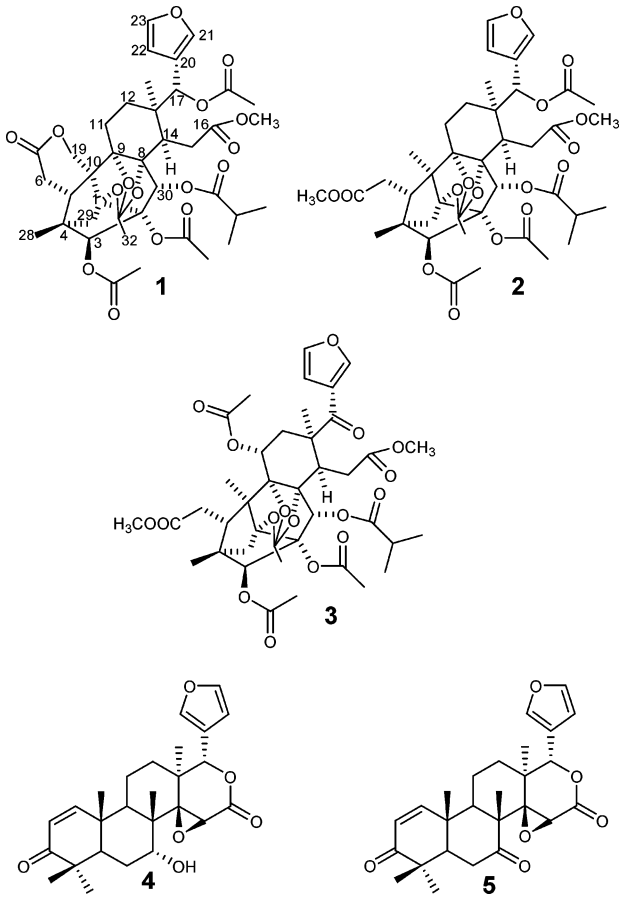
The dicholoromethane extract of *Pseudocedrela kotschyi* root demonstrated marked antileishmanial properties in preliminary screening of extracts from 21 species commonly used in Malian traditional medicine. Phytochemical investigation of the active extract yielded three novel phragmalin-type limonoid orthoacetates (**1**−**3**), named kotschyins A−C, and the known compounds 7-deacetylgedunin (**4**) and 7-deacetyl-7-oxogedunin (**5**). The structures of **1**−**3** were elucidated by analytical methods including 1D- and 2D-NMR spectroscopy together with MS spectroscopy. The relative configurations of **1**−**3** were assigned on the basis of NOE correlations. The extract and the isolated compounds were tested for their antiprotozoal activities against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Plasmodium falciparum* as well as for cytotoxicity toward the L-6 cell line. The crude extract and the two gedunin derivatives exhibited good in vitro activity against all of these parasites.

Pseudocedrela kotschyi (Schweinf.) Harms (Meliacae), previously known as *Cedrela kotschyi* (Schweinf.) Harms and *Pseudocedrela chevalieri* C. DC., is a 6- to 12-meter-tall tree irregularly distributed in bushy and woody savannahs from Senegal to Sudan.[1] Compounds previously isolated from *P. kotschyi* include limonoids, the derivatives 7-deacetyl-7-oxogedunin, 7-deacetylgedunin,[2] and pseudrelone B.[3,4] *P. kotschyi* is commonly used in the sub-Saharan region to treat various diseases. Indeed, traditional preparations of bark macerates are employed topically by Manding people in the treatment of yaws and syphilis chancres.[1] *P. kotschyi* is also used in the Kudana State (Nigeria) to treat sleeping sickness,[5] as well as in Sudan[6] and Mali[7] to treat malaria. In a preliminary antileishmanial screening of plants used in Malian traditional medicine, the CH_2Cl_2 extract of *P. kotschyi* roots was found to display marked activity against the intracellular form of *L. major* (5.6 ± 1.2% of survival following exposure to 35 μg/mL) without showing any toxicity to cells from the RAW macrophagic line.[8] This result motivated phytochemical investigation in order to isolate the substances responsible for its leishmanical properties. We report herein the isolation of five compounds and the in vitro antiprotozoal activities of some of them on *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Plasmodium falciparum* strains.

Results and Discussion

A preliminary LC/UV/APCI-MS analysis was performed on the active CH_2Cl_2 extract of *P. kotschyi* roots in order to gather structural information on its content. Major UV-active compounds displaying ions of *m*/*z* 439, 441, and 291 were isolated after elution of the extract on open column chromatography and subsequent purification using reversed-phase MPLC and semipreparative HPLC techniques. A comparison of the experimental measurements obtained by NMR and MS spectroscopies with data reported in literature allowed us to associate the *m*/*z* 441 ion with 7-deacetylgedunin and the *m*/*z* 439 ion with 7-deacetyl-7-oxogedunin,[9] two limonoid derivatives already characterized from the wood of *P. kotschyi*.[2] Besides known compounds, another set of interest was detected as ions of *m*/*z* between 770 and 800 on the LC/UV/MS chromatogram, and this led to the isolation of compounds **1**, **2**, and **3**.



High-resolution mass spectrometry of **1** gave an ion peak $[M + Na]^+$ at *m*/*z* 795.2834 corresponding to a molecular formula of $C_{39}H_{48}O_{16}Na$ and 16 double-bond equivalents. According to the ^1H, ^{13}C, HMQC, and HMBC NMR spectra (Table 1), **1** was shown to possess eight tertiary methyl groups (including three acetyls, two methyls, one methoxy, and one isopropyl), three methine protons

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Table 1. NMR Data of Kotschyins A and B (**1**, **2**) (in $CDCl_3$)

	1					**2**			
pos.	^{1}H	^{13}C	HMBC[a] (H→C#)	NOESY[a] (H→H#)	pos.	^{1}H	^{13}C	HMBC[a] (H→C#)	NOESY[a] (H→H#)
1		86.4			1		85.5		
2		86.1			2		86.7		
3	5.18 (s)	81.5	1, 3-O*C*O, 4, 5, 28, 29, 30	29$_{pro-S}$	3	5.13 (s)	81.4	2, 3-O*C*O, 4, 5, 28,	28, 29 $_{pro-S}$
4		46.3			4		46.3		
5	2.56 (m)	33.2	1, 3, 6, 7, 19, 29	30	5	2.88 (m)	35.7	3, 19	30
6	2.38 (dd, 17.6, 5.4)	31.2	5, 7, 10		6	2.41 (m)	34.0	4, 5, 7	
	2.57 (m)					2.20 (m)			
7		171.6			7		172.5		
8		86.7			8		86.5		
9		85.4			9		87.2		
10		45.4			10		46.0		
11α	2.16 (m)	26.2	8, 10, 12, 13		11α	1.94 (m)	25.8	1, 8	
11*β*	1.89 (m)				11*β*	1.71 (m)		13, 12, 9	
12α	1.02 (br d, 12.7)	31.8	9, 11, 18		12α	0.84 (m)	31.8	9, 17, 18	
12*β*	1.20 (m)			17, 30, 5, 11*β*	12*β*	1.32 (m)			17, 30
13		39.3			13		39.4		
14	2.33 (dd, 3.4, 9.8)	47.7	30	18	14	2.38 (m)	47.8	9, 13, 15, 16, 30	
15	2.85 (dd, 3.4, 16.1)	31.2	8, 13, 14, 16		15	2.89 (m)	31.5	8, 13, 14, 16	
	2.24 (dd, 16.1, 9.8)		8, 13, 14, 16	17		2.27 (m)			17
16		174.3			16		174.7		
17	5.68 (s)	70.5	13, 14, 17-O*C*O, 20, 22, 23	30	17	5.77 (s)	71.2	18, 13, 14, 22, 20, 23, 17-O*C*O	
18	1.22 (s)	21.6	12, 13, 14, 17	22	18	1.16 (s)	21.2	12, 13, 14, 17	14, 22
19	4.76 (d, 13.7)	69.2	5, 7, 9, 10	29$_{pro-R}$	19	1.11	16.7	1, 5, 9, 10	11α, 29 $_{pro-R}$
	4.33 (d, 13.7)		9, 10						
20		122.6			20		123.3		
21	7.37 (br t, 1.5)	143.3	20, 22, 23	18	21	7.33 (br t, 1.5)	142.8	20, 22, 23	
22	6.39 (br d, 2.0)	109.5	20, 21, 23		22	6.44 (br d, 2.0)	109.8	20, 21, 23	
23	7.68 (br s)	142.3	20, 21, 22		23	7.64 (br s)	141.6	20, 21, 22	
28	0.94 (s)	14.0	3, 4, 5, 29	3, 29	28	0.88 (s)	14.9	3, 4, 5, 29	
29 $_{pro-R}$	2.28 (d, 11.2)	39.3	1, 3, 4, 28		29 $_{pro-R}$	1.94 (d, 11.2)	40.5	10, 3, 1, 2	
29 $_{pro-S}$	1.69 (d, 11.2)		2, 3, 5, 10		29 $_{pro-S}$	1.63 (m)			
30	6.08 (s)	69.5	3, 8, 30-O*C*O		30	6.28 (s)	70.0	1, 3, 30-O*C*O	17
31		119.6			31		119.0		
32	1.66 (s)	21.0	31		32	1.64 (s)	21.2	31	
16-OMe	3.69 (s)	51.8	16		16-OMe	3.68 (s)	51.7	16	
17-OAc		169.3			17-OAc		169.2		
	1.94 (s)	21.5	17-O*C*O			1.95 (s)	21.5	17-O*C*O	
3-OAc		169.9			3-OAc		170.6		
	2.27 (s)	21.6	3-O*C*O,			2.33 (s)	21.5	3-O*C*O	
2-OAc		170.3			2-OAc		170.2		
	2.13 (s)	22.1	2, 2-O*C*O			2.12 (s)	22.2		
30-OiBut		174.4			7-OMe	3.61 (s)	52.0	7	
	2.6 (m)	34.5	*C*H$_3$-iPr, 30-O*C*O		30-OiBut		174.2	30-OCOiPr	
	1.25 (d, 7.3)	18.4	*C*H$_3$-iPr, 30-O*C*O, *C*H-iPr			2.56 (m)	34.5	*C*H$_3$-iPr, *C*H3-iPr, 30-O*C*O	
	1.22 (d, 7.3)	17.8	*C*H$_3$-iPr, *C*H-iPr			1.25 (s)	18.6	*C*H$_3$-iPr, 30-O*C*O	
						1.22 (s)	18.1	*C*H$_3$-iPr, 30-O*C*O	

[a] Main observed HMBC and NOESY correlations are presented.

attached to a carbon adjacent to an oxygen atom (δ_H 5.18, 5.68, 6.08), six methylenes, and three downfield shifted proton signals attributed to a 3-substituted furan ring (δ_H 7.37, 6.39, 7.68 connected to carbons at δ_C 143.3, 109.5, 142.3, respectively, according to the HSQC). These data suggested that **1** has a methyl meliacate skeleton typical of limonoids of the Meliaceae family.[10] The ^{1}H signal at δ_H 5.68 was assigned to H-17 by correlations observed in the HMBC spectrum with the carbon signals of the furan ring. Long-range ^{1}H−^{13}C correlations were observed between H-17 and a quaternary carbon at δ_H 39.3 (C-13), correlating to the methylene protons H-15 (δ_H 2.85, 2.24) and H-11 (δ_H 2.16, 1.89) as well as to a tertiary methyl group at H-18 (δ_H 1.22) in the HMBC spectrum. The ^{1}H NMR resonance δ_H at 5.68 (H-17) also displayed long-range ^{1}H−^{13}C correlations with an acetoxy carbonyl moiety (δ_C 169.3, 17-O*C*O) and a carbomethoxy signal at δ_H 3.69 (16-O*C*H$_3$) ^3J correlated to the 16-ester carbonyl (δ_C 174.3). The long-range correlations observed between H-15 methylene protons and C-16 confirmed their α-position to this carbonyl function. Regarding the HMBC correlations of the methylenic protons H-29 (δ_H 2.28, 1.69, d, J = 11.2 Hz) with C-1, C-3, C-4, C-5, and C-10 (δ_C 86.4, 81.5, 46.3, 33.2, and 45.4 respectively) and the absence of a 1-ketonic group, it could be suggested that (as in the case of natural

bicyclononanes of the methyl meliacate group) the compound is a 1,29-cyclomeliacate.[10] Long-range $^1H-^{13}C$ correlations observed between the H-29 methylene protons and the tertiary methyl signal at C-28, the methine signal at C-3, and the quaternary carbon C-4 indicated the presence of a 4,29,1-bridge characterized by the C-29 resonance at δ_C 39.3.[11] This suggested **1** to be a ring D-*seco* phragmalin-type limonoid derived from mexicanolide. Similarly to swietenialides isolated from *Swietenia mahogany* JACQ,[12] the presence of a quaternary carbon at δ_C 119.6 (C-31) showing a HMBC correlation with the H-32 signal (δ_H 1.66) suggested the presence of an orthoacetate moiety.[11] Previously reported phragmalin derivatives usually have orthoacetate groups at positions 1, 8, 9 or 8, 9, 14 or 8, 9, 30.[12,13] The observation of long-range $^1H-^1H$ COSY correlations between ^1H NMR signals at δ_H 2.33 (H-14) and δ_H 2.85 (also δ_H 2.24) (H-15) together with the long-range $^1H-^{13}C$ correlation between H-30 (δ_H 6.08) and a carbonyl signal at C-30 confirmed the location of the orthoacetate moiety on positions 1, 8, 9. As in the case of tabulalide A isolated from *Chukrasia tabularis*,[14] an oxymethylene group (δ_C 69.2; δ_H 4.33 and 4.76, each d, J = 13.7 Hz) was observed in the ^{13}C and ^1H NMR data. It was correlated, in the HMBC spectrum, to a lactonic carbonyl carbon at δ_C 171.6 (C-7), which implied the presence of a six-membered 5−10 lactone ring. Note that the signal at δ_C 39.3 showed long-range correlations with H-3, H-5, H-11, H-15, H-17, H-18, and H-28 signals (δ_H, 5.18, 2.56, 2.16 and 1.89, 2.85 and 2.24, 5.68, 1.22, and 0.94, respectively), suggesting that the resonances for C-13 and C-29 are superimposed. Finally, ^1H, ^{13}C NMR and HSQC spectra exhibited signals of three acetyl groups (δ_H 1.94, 2.27, 2.13, 3H each s; δ_C 21.5, 21.6, 22.1 and 169.3, 169.9, 170.3 for carbonyl groups, respectively) and one isobutyrate group (δ_H 2.6, 1.25, 1.22; δ_C 34.5, 18.4, 17.8, respectively, and δ_{CO} 174.4). According to the HMBC spectrum, the oxymethine protons at δ_H 5.18 (H-3) showed a ^3J correlation with the carbonyl carbon signal at δ_C 169.9 (3-O*C*O) and H-29 (δ_H 2.28 and 1.69) exhibited a ^3J long-range correlation with the quaternary carbon signal at δ_C 86.1 (C-2), which was correlated to the methyl proton signal at δ_H 2.13. Similarly, correlation of the methine proton H-30 with the isobutyryl carbonyl carbon signal at δ_C 174.4 indicated the presence of an isobutyrate moiety on C-30. NOESY experiments were conducted using a molecular model in order to elucidate the stereochemistry of **1**. Cross-peaks of the ^1H NMR signal at δ_H 1.20 (H-12) with the ones at δ_H 2.56 (H-5), 5.68 (H-17), and 6.08 (H-30) indicated a β-orientation for these four protons and the folded conformation of **1**. The 29-methylene proton signals at δ_H 1.69 (*pro-S*) and 2.28 (*pro-R*) showed NOE correlations with the ^1H NMR signal at δ_H 0.94 (H-28). They also specifically correlated with H-3 (for 29 *pro-S*) and H-19 (for 29 *pro-R).* This observation clarified the relative stereochemistry between these protons in the tricyclo-[3.3.1.1]decane ring system. As observed in the molecular model, the presence of a 1,8,9-orthoacetate moiety indicated a boat conformation for ring B. The relative configuration of **1** was thus found to be identical to that of swietenialides[12] and tabulalides[14] isolated from the genera *Swietenia* and *Chukrasia*, respectively. It is of interest that both genera are closely related botanically to the *Pseudocedrela* genus. The relative configuration determined for **1** was confirmed by the −55° optical rotation value for kotschyin A (**1**) of sign similar to those of swetenialide D (−26°),[12] leandreanin C (−30°),[15] or tabulalide D (−52°).[14]

High-resolution mass spectrometry of **2** gave an $[M + Na]^+$ ion peak at *m*/*z* 811.3147 corresponding to a molecular formula of $C_{40}H_{52}O_{16}Na$. Comparison of ^1H NMR spectra of **1** and **2** showed that the latter lacked the two coupled doublets at δ_H 4.76 and 4.33, which were connected to the same carbon at δ_C 69.2 (C-19) in **1**. Instead, a methyl group signal was seen as an additional singlet at δ_H 1.11. This suggested that the oxymethylene group in **1** was replaced by a tertiary methyl group (Me-19) in **2**. This was confirmed by the long-range correlations of methoxy protons at δ_H 3.61 (δ_C 52.0, 7-O*C*H$_3$) with the carbonyl at δ_C 172.5 (C-7). All other ^1H and ^{13}C signals and 2D correlations were in agreement with **1**. The structure of **2** was similar to that of **1** but had an open δ-lactonic ring between positions 5 and 10. NOESY correlations observed for **2** confirmed its stereochemistry to be identical to that of **1** according to data presented in Table 1.

A pseudomolecular $[M + Na]^+$ ion was seen for **3** at *m*/*z* 825.2940 in the HRESIMS spectrum corresponding to a molecular formula of $C_{40}H_{50}O_{17}Na$. The UV spectrum of **3** featured an extra absorption band at 255 nm in addition to those at 199 and 218 nm observed for **1** and **2**. In addition to characteristic structural moieties such as the orthoacetate group and the 4,29,1-bridge, the ^{13}C NMR spectrum indicated the presence of one apparent keto group at δ_C 199.4. The ^1H NMR signal at H-21 corresponding to the α-furyl moiety experienced a downfield shift at δ_H 8.06. The ^1H NMR spectrum of **3** also lacked the δ_H 5.68 proton signal observed for **1** (Table 2). This suggested the presence of a C-17 carbonyl group such as in pseudrelone B isolated from *P. kotschyi*[3] or in leandreanin isolated from *Neobeguea leandreana*.[15] The additional maximum in the UV spectrum of **3** could also be explained by the presence of the ketonic function. Another oxymethine signal was observed at δ_H 5.47 (H-11) featuring a long range $^1H-^{13}C$ correlation with an acetoxy carbonyl group at δ_C 170.1 (11-O*C*O). A cross-peak observed in the COSY spectrum between this latter signal and H-12 (δ_H 2.37 and 2.49) allowed us to place the acetoxy group at C-11. This was confirmed by the downfield shift of C-11 from about δ 26.0 in **1** and **2** to δ 67.1 in **3**. Following these results, compound **3** was characterized as a novel phragmalin-type limonoid derivative, named kotschyin C.

The Meliaceae family is one of the top 5 families in terms of traditional use against malaria. Antiparasitic activities of the compounds for which a sufficient amount of material was available were evaluated, as well as the crude extract (see Table 3). The limonoid derivatives 7-deacetylgedunin (**4**) and 7-oxo-7-deacetylgedunin (**5**) displayed a low-range micromolar IC_{50} against *P. falciparum*, *T. b. rhodesiense*, and the axenic form of *L. donovani*. Such results can explain the antiparasitic properties of the raw extract. It was also observed that 7-deacetylgedunin showed moderate activity against *T. cruzi*, in contrast to 7-deacetyl-7-oxogedunin, which was inactive in the same test. Kotschyin A (**1**) was inactive in the antiprotozoal assays. Some limonoid compounds are known for their good insect antifeedant activity.[14,19] However, this is the first report concerning the testing of specific orthoacetate derivatives demonstrating that they are inactive in vitro on *P. falciparum*, *T. b. rhodesiense*, *T. cruzi*, and *L. donovani* organisms. In conclusion, the raw extract and the two gedunin derivatives exhibited good in vitro activity against all of these parasites, suggesting a lack of specificity for a protozoal target.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Perkin-Elmer 241 polarimeter (MeOH, *c* in g/100 mL). UV spectra were measured in MeOH on a Perkin-Elmer Lambda 20 spectrophotometer. IR spectra were measured with a FTIR spectrometer Spectrum One B (Perkin-Elmer). ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer (500 and 125 MHz, respectively) in $CDCl_3$: chemical shifts in ppm as δ relative to Me_4Si (internal standard). LC-APCIMS was performed using a Finnigan MAT (San Jose, CA) ion trap mass spectrometer equipped with a Finnigan interface operated under the following conditions: positive ion mode; capillary voltage, 19 V; capillary temperature, 150 °C; source voltage, 6 kV; sheath gas, nitrogen at 65 psi. HRESIMS were recorded on a FTMS 4.7T BioApex II (Bruker) spectrometer using electrospray as the ion source, positive mode. Open column chromatography was performed using silica gel 60 AC-C (70−200 μm; Merck). Analytical HPLC was carried out on a HP 1100 system equipped with a photodiode array detector (Agilent Technologies). Extracts and fractions were analyzed on a Symmetry C_{18} column (4 μm; 4.6 × 250 mm; Waters)

Table 2. NMR Data of Kotschyin C (**3**) (in $CDCl_3$)

pos.	^1H	^{13}C	HMBC[a] (H→C#)	NOESY[a] (H→H#)
1		85.9		
2		85.8		
3	5.04 (s)	80.7	1, 3-O*C*O, 4, 5, 6, 28, 29, 30,	28, 29_{pro-S}
4		46.0		
5	2.67 (m)	35.7	1, 3, 4, 6, 19, 7	12β, 30
6	2.40 (m)	34.3	5, 4, 7	
	2.30 (m)			
7		172.1		
8		86.6		
9		85.5		
10		45.6		
11	5.47 (s)	67.1	8, 11-O*C*O, 13	12β, 18, 19
12α	2.37 (m)	35.9	13, 17, 18	
12β	2.49 (m)			5, 30, 11
13		47.1		
14	3.33 (m)	44.5	18, 17, 15, 30,	18
15	3.25 (dd, 21.0, 7.8)	30.3	8,13, 14, 16	3.33 (dd, 21.0, 7.8)
16		175.9		
17		199.4		
18	1.50 (s)	29.0	12, 13, 14, 17	
19	1.24 (s)	16.2	1, 5	29_{pro-R}20126.1
21	8.06 (br s)	146.5	20, 22, 23	18
22	6.80 (br d, 1.0)	111.1	20, 21, 23	18
23	7.42 (br t, 1.5)	142.4	20, 21, 22	
28	0.83	14.6	3, 4, 5, 29	3
29_{pro-R}	1.94 (d, 11.2)	40.1	2, 3, 4, 28	
	1.60 (d, 11.2)			
29_{pro-S}			2, 3, 5, 10	
30	5.71 (s)	68.3	2, 3, 30-OCO	
31		118.9		
32	1.60 (s)	20.3	*31*	
16-OMe	3.71 (s)	51.8	16	
11-OAc		170.1		
	2.07 (s)	20.8	11-O*C*O	
3-OAc		169.7		
	2.20 (s)	21.0	3-O*C*O	
2-OAc		169.6		
	2.09 (s)	21.9	2-O*C*O	
30-OiBut		172.3		
	2.53 (m)	34.0	*C*H_3-iPr, 30-O*C*O	
	1.31 (d, 6.8)	18.4	*C*H-iPr, *C*H_3-iPr, 30-O*C*O	11
	1.20 (d, 6.8)	18.5		
			*C*H-iPr, *C*H_3-iPr, 30-O*C*O	

[a] Main observed correlations are presented.

Table 3. Antiprotozoal Activities and Cytotoxicity of CH_2Cl_2 Extract and Pure Compounds from the Roots of *P. kotschyi* (IC_{50} values in μg/mL)

	T. brucei rhodesiense	*T. cruzi*	*L. donovani axen.*	*P. falciparum*	cytotoxicity
CH_2Cl_2 extract	8.89	13.50	7.36	3.93	65.41
7-deacetylgedunin	3.45	5.20	1.4	1.36	13.3
kotschyin A (**1**)	11.60	>30	>30	>5	>90
7-deacetyl-7-oxogedunin	3.40	>30	0.994	1.775	>90
reference drug	melarsoprol	benznidazol	miltefosin	chloroquin	podophyllotoxin
	0.004	0.74	0.12	0.023	0.005

using a CH_3CN-H_2O gradient with 0.2% acetic acid (plates of 10 min at 50:50, 52:48, 54:46 and up to 60:40 in 10 min and to 100:0 in 10 min) in 60 min. The detection was performed at 202 nm. MPLC separations were done with a Büchi 681 pump equipped with a Knauer UV detector using a Lichroprep RP-18 column (40−63 μm; 460 × 15 mm i.d.; Merck). LPLC was done on Lobar RP-18 columns (LiChroprep 40−63 μm, 310 × 25 mm i.d.; Merck) using a cfg ProMinent Duramat pump equipped with a Bromma 2238 Uvicord SII detector. Semi-preparative HPLC was performed with a LC-8 pump equipped with a SPD-10A VP (Shimadzu) detector using a μ-Bondapak C_{18} prepacked radial-compression column (10 μm, 25 × 100 mm; Waters).

Plant Material. *Pseudocedrela kotschyi* roots were collected at the Médine Market (Bamako, Mali) and identified by D. Diallo. A voucher specimen (2000038) is deposited at the Laboratory of Pharmacognosy and Phytochemistry at the University of Geneva.

Extraction and Isolation. Dried roots were successively extracted at room temperature with CH_2Cl_2, MeOH, and H_2O (3 times 24 h at room temperature for each solvent). After filtration, extracts were evaporated under reduced pressure and lyophilized to yield 17.0 g of CH_2Cl_2 extract, 45.3 g of MeOH extract, and 1.7 g of aqueous extract. An amount of 12 g of the CH_2Cl_2 extract was subjected to open CC over silica gel using a hexane−EtOAc gradient (16:1 to 1:2) to give 175 fractions, from which 7-deacetylgedunin (**4**) (fractions 96 to 98, 162 mg) was obtained. Fractions 122 to 126 (751 mg) were regrouped and chromatographed over SiO_2 to give 118 fractions, of which 30 to 40 (109 mg) were purified by μ-Bondapak C_{18} semipreparative HPLC (MeOH−H_2O isocratic, 57:43), affording 7-deacetyl-7-oxogedunin (**5**) (28 mg). Fractions 51 to 58 (302 mg) were separated further by MPLC RP-18 with a MeOH−H_2O gradient (55:45 to 100:0), yielding **1** (20 mg). Fractions 127−133 (810 mg) were subjected to MPLC over RP-18 to give **2** (7 mg) and **3** (5 mg). Finally, fractions 172 to 175 (701 mg) were subjected to MPLC over RP-18 using MeOH−H_2O (gradient, 20:80 to 100:0), yielding (−)-catechin (55 mg) and (−)-epicatechin (39 mg).

Antiplasmodial Assay. Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). The test was done as described before.[20]

***T.b. rhodesiense, T. cruzi, Leishmania donovani* Axenic Amastigote and Cytotoxicity Assays.** All of these assays were as described by Tasdemir et al.[21]

Kotschyin A (1): amorphous, white powder; $[\alpha]_D$ −55 (MeOH, *c* 0.1); UV (MeOH, *c* 2.5 × 10^{-5}) λ_{max} (log ϵ) nm 199 (4.08), 218 (sh); IR ν_{max} (KBr) cm^{-1} 3466, 2955, 1750, 1377, 1244; NMR data, see Table 1; HRESIMS *m*/*z* 795.2834 (calcd for $C_{39}H_{48}O_{16}Na$ 795.2840).

Kotschyin B (2): amorphous, white powder; $[\alpha]_D$ −60 (MeOH, *c* 0.1); UV (MeOH, *c* 2.5 × 10^{-5}) λ_{max} (log ϵ) nm 199 (4.06), 218 (sh); NMR data, see Table 1; HRESIMS *m*/*z* 811.3147 (calcd for $C_{40}H_{52}O_{16}Na$ 811.3153).

Kotschyin C (3): amorphous, white powder; $[\alpha]_D$ −17 (MeOH, *c* 0.1); UV (MeOH, *c* 2.5 × 10^{-5}) λ_{max} (log ϵ) nm 199 (4.23), 218 (sh), 255 (3.32); NMR data, see Table 2; HRESIMS *m*/*z* 825.2940 (calcd for $C_{40}H_{50}O_{17}Na$ 825.2945).

7-Deacetylgedunin (4): amorphous, white powder; $[\alpha]_D$ −132 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ϵ) nm 217 (4.09), 199 (4.13); APCIMS *m*/*z* 441 $[M + H]^+$.[22]

7-Deacetyl-7-oxogedunin (5): amorphous, yellow powder $[\alpha]_D$ −30 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ϵ) nm 217 (4.29), 199 (4.27); APCIMS *m*/*z* 439 $[M + H]^+$.[22]

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