Two New Cytotoxic Compounds from *Tapirira guianensis*

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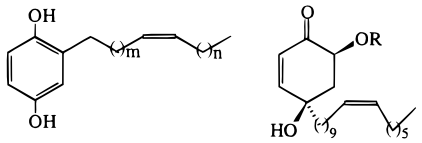
Two new cytotoxic compounds, 2-[10(*Z*)-heptadecenyl]-1,4-hydroquinone (**1**) and (4*R*,6*R*)-dihydroxy-4-[10(*Z*)-heptadecenyl]-2-cyclohexenone (**2**) have been isolated from a MeOH extract of seeds of *Tapirira guianensis*. The structures were established through spectral analysis of the isolates and their derivatives.

The genus *Tapirira* (Anacardiaceae) is composed of approximately 15 species found from Mexico throughout South America. *T. guianensis* Aubl. is a tall tree, usually occurring in the Atlantic forest of Brazil. This plant is commonly known as "pau-pombo" whose bark and leaves are used by the local population against leprosy, diarrhea, and syphilis.[1,2] A search of the NAPRALERT database revealed that an aqueous EtOH extract of the dried entire plant had previously shown cytotoxic and general toxic effects[3] and that the H_2O extract of the stembark had shown uterine stimulant effects.[4] There are no reports in the literature regarding the chemical constituents of this plant.

As part of a continuing program aimed at the discovery of novel anticancer agents, it was found that the $CHCl_3$ extract of the seeds of *T. guianensis* displayed cytotoxic activity against human prostate cancer (LNCaP, Table 1). Bioactivity-directed fractionation of the extract afforded, in addition to β-sitosterol, two new cytotoxic compounds, 2-[10(*Z*)-heptadecenyl]-1,4-hydroquinone (**1**) and (4*R*,6*R*)-dihydroxy-4-[10(*Z*)-heptadecenyl]-2-cyclohexenone (**2**).



1. m = 8, n = 5 **2**. R = H

3. m = 6, n = 7 **4**. R = (4-Cl)-C_6H_4-CO-

Compound **1** exhibited the molecular formula $C_{23}H_{38}O_2$ as established by HREIMS (obsd 346.2858, calcd 346.2872) and its ^{13}C and ^1H NMR spectra. Analysis of the ^1H NMR indicated a 1,2,4-trisubstituted aromatic ring (1, 4 = OH; 2 = alkenyl chain). Comparison of the ^{13}C NMR data of the aromatic ring with lanneaquinol (**3**) and 4-heptadecyl-1,2-dihydroxybenzene[5] indicated a structure similar to lanneaquinol (2-alkenyl-1,4-dihydroxybenzene) for **1**. In support of this notion, the FLOCK experiment showed correlations for the resonance at δ 2.54 (H-1′) with C-1 (δ 147.33), C-2 (δ 130.13), and C-3 (δ 116.84). Comparison of the chemical shifts observed in the ^{13}C NMR for C-10′ and C-11′ and the coupling constants displayed in the ^1H NMR for H-10′ and H-11′ of **1** with the data for (*Z*)- and (*E*)-straight-chain esters[6] allowed the assignment of the (*Z*)-stereochemistry to the side-chain double bond in the alkenyl chain. The structural difference between **1** and lanneaquinol (**3**) was the position of the double bond. This was established through the MS of the α,β-bis(methylthio) derivative produced by the reaction of the 2-(heptadec-10(*Z*)-enyl)hydroquinone diacetate derivative with dimethyl disulfide.[7] Thus, the fragments observed at *m/z* 379 ($C_{21}H_{31}O_4S$) and *m/z* 145 ($C_8H_{17}S$) permitted establishment of the double bond at carbons 10′ and 11′ in **1**.

Table 1. Cytotoxicity of the $CHCl_3$ Extract of Seeds of *Tapirira guianensis* and of Isolates **1** and **2** (IC_{50} in μg/mL)

	cell line[a]						
sample	BC1	Lu1	Col2	KB	KB-V (+VLB)	KB-V (−VLP)	LNCaP
$CHCl_3$ extract	>20	>20	>20	>20	>20	>20	5.2
1	1.3	0.3	0.8	0.5	0.5	0.6	0.2
2	4.3	4.4	1.8	1.5	4.1	4.2	0.3

[a] BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human epidermoid carcinoma; KB-V1 (+VLB) = vinblastine (VLB)-resistant KB with 1 μg/mL VLB; KB-V1 (−VLB) = VLB-resistant KB without VLB; LNCaP = human hormone-dependent prostate cancer.

4,6-Dihydroxy-4-[10(*Z*)-heptadecenyl]-2-cyclohexenone (**2**) was obtained as a transparent oil. The molecular formula was established as $C_{23}H_{40}O_3$ through a $[M - 1]^+$ peak at *m/z* 363 displayed in the negative FABMS and by analysis of the ^1H and ^{13}C NMR spectral data. The IR spectrum showed a broad OH absorption band at 3394 cm^{-1} and the absorption of a carbonyl group at 1680 cm^{-1}, indicating an α,β-unsaturated ketone. This observation was supported by the ^1H NMR spectrum which showed peaks for two protons in a C=C bond conjugated to a carbonyl group in a cis relationship (J = 10.2 Hz), i.e., H-2 (δ 6.02) and H-3 (δ 6.89) appeared as a doublet and a double doublet, respectively. In addition to these signals, the ^1H NMR spectrum exhibited resonances for alkenyl chain, methylene, and oxymethine protons. The ^{13}C NMR spectrum corroborated the presence of the above groups and showed an additional resonance for a quaternary oxygenated carbon at δ 74.59 (Table 2). The FLOCK experiment permitted location of one hydroxyl group at C-6, as well

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Table 2. ^{1}H NMR,[a] and ^{13}C NMR[b] Data and Long-Range Correlations of Compounds **1** and **2**

	1			**2**		
position	H	C	FLOCK	H	C	FLOCK
1		147.33			200.88	
2		130.13		6.02 (*d*; 10.6)	126.58	74.59, 64.04
3	6.63 br *s*	116.84	31.81	6.89 (*dd*; 10.6, 2.7)	150.33	200.88, 41.17
4		149.25			74.59	
5	6.55 (*dd*; 2.7, 8.7)	113.28	116.84, 147.83		41.17	74.59, 64.04
5_{ax}				2.12 (*dd*; 5.5, 13.0)		
5_{eq}				2.28 (*ddd*; 4.5, 13.0, indt)		
6	6.64 (*d*; 8.7)	116.04	130.13, 113.28, 149.25	4.70 (*dd*; 4.5, 5.5)	64.04	150.33
1′	2.54 (*t*; 8.1)	31.81	147.33, 116.84, 130.13	1.77 (*t*; 9.9)	38.88	74.59
2′		31.96		1.2−1.8		
3′−8′	1.2−1.6	29.0−29.8		1.2−1.8		
9′, 12′	1.2−1.6	27.25		2.01 *m*	27.21	
10′, 11′	5.3 (*t*; 4.8)	129.96, 129.97	27.25	5.34, (*t*; 4.3)	129.90, 129.84	27.21
17′	0.87 (*t*; 5.8)	14.13		0.89 (*t*; 5.9)	14.13	

[a] 300 MHz, $CDCl_3$, δ (ppm), m and J (Hz). [b] 75 MHz.

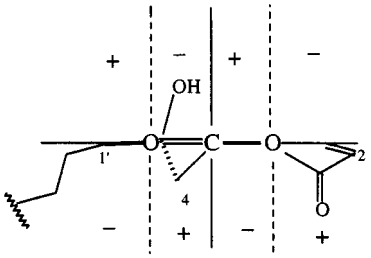

Figure 1. The benzoate sectors for carbons of the 4-chlorobenzoyloxy derivative of **2**.

as the alkenyl chain and one hydroxyl group at C-4, due to the correlations observed between H-3 (δ 6.89) with C-1 (δ 200.88), C-5 (δ 41.17), and C-1′ (δ 38.88) and between the resonance at δ 6.02 (H-2) with C-4 (δ 74.59) and C-6 (δ 64.04). The COSY spectrum showed a correlation between H-3 and H-5 due to a **W** coupling. The pseudoaxial position of the hydroxyl group at C-6 was suggested by the coupling constants observed for H-5 with H-6_{ax} (J = 5.5 Hz) and H-6_{eq} (J = 4.5 Hz). The alkenyl chain was located in a pseudoequatorial orientation on the basis of the NOESY spectrum, which showed correlations between H-5 and H-1′. Similar to **1**, the isolate **2** possessed a double bond in the C_{17} side chain with a *Z*-stereochemistry. Analysis of the FABMS of **2** permitted the double bond to be established at C-10′ through the fragment ion at *m*/*z* 279 derived from cleavage between C-11 and C-12. The FABMS of the α,β-bis(methylthio) derivative of **2** confirmed the above statement, showing two intense fragments ions at *m*/*z* 313 ($C_{17}H_{29}O_3S$) and *m*/*z* 145 ($C_8H_{17}S$). The absolute configuration of **2** was determined by application of the aromatic chirality method for secondary cyclic alcohols.[8] When compound **2** was submitted to 4-chlorobenzoylation, the monobenzoate derivative **4** displayed a CD spectrum with a positive Cotton effect at 243 nm. This signal represented the positive chirality between the long axes of the carbonyl β,δ-double bond and the 4-chlorobenzoate chromophores (Figure 1). Thus, the OH group at C-6 should be fixed in a β-position permitting assignment of the 4*R*,6*R* absolute configuration for **2**.

The isolates were evaluated for their cytotoxic activity against a panel of human cancer cell lines.[9] Both isolates were broadly active, with **1** showing IC_{50} values in the range 0.2−1.3 μg/mL. Compound **2** was most active in the LNCaP prostate cancer cell line (Table 1). The mechanism of action of these compounds is presently under investigation.

Experimental Section

General Experimental Procedures. The UV spectra were recorded on a Beckman model DU-7 spectrophotometer. The FTIR spectrum was recorded on a MIDAC spectrophotometer. Optical rotation was determined on a Perkin-Elmer model 241 polarimeter. CD spectra were recorded on a JASCO J710 automatic spectropolarimeter. ^{1}H and ^{13}C NMR, APT, SFORD, COSY, HETEROCOSY, and FLOCK spectra were obtained on either a Varian XLS 300 or a Bruker AM-400 instrument employing $CDCl_3$ as solvent and reference. The MS were recorded on a Finnigan MAT 90 spectrometer. Column chromatography was carried out on Si gel 60, the fractions were monitored by TLC on Si gel, and spots were revealed with anisaldeyde/H_2SO_4 reagent and UV light (254 nm).

Plant Material. The seeds of *T. guianensis* Aubl. were collected in August 1995 on the Campus Universitário de Ondina, Universidade Federal da Bahia, Salvador (BA), Brazil. The plant material was identified by Professor Maria Lenise S. Guedes, and a voucher was deposited at the Herbarium Alexandre Leal Costa of the Universidade Federal da Bahia under acquisition no. 028377.

Extraction and Isolation. After being dried in a ventilated oven (45 °C), the seeds (400 g) were ground and extracted with petroleum ether. The resulting extract (32.9 g) was partitioned between hexane and MeOH/H_2O (9:1). The MeOH phase was submitted to cytotoxicity bioassays and showed activity against the LNCaP cell line (IC_{50} = 5.2 μg/mL).

The MeOH (10.5 g) extract was submitted to column chromatography over Si gel (150 g) eluted with $CHCl_3$/EtOAc (9:1, 4:1, 7:3, and 1:1). Fractions of 100 mL were recovered and further combined into four new fractions on the basis of TLC monitoring. Fraction 1 (6.4 g) was the main fraction and was shown to be a mixture of unsaturated fatty acids. The second and fourth fractions showed strong cytotoxicity activity. The second fraction (418 mg) was submitted to column chromatography on Si gel eluted with $CHCl_3$:EtOAc (9:1) to afford the pure compound **1** (88.8 mg, 0.022%), in addition to β-sitosterol (95.1 mg, 0.024%). The fourth fraction (1.73 g) afforded the pure compound **2** (743.9 mg, 0.19%) after

column chromatography over Si gel eluted with $CHCl_3$/MeOH (49:1) and followed by permeation on Sephadex LH 20 using MeOH/$CHCl_3$ (4:1) as eluent.

2-[10(*Z*)-heptadecenyl]-1,4-hydroquinone (1): wax; HRFABMS *m/z* 346.2858 (calcd for $C_{23}H_{38}O_2$, 346.2872); FABMS *m/z* 346 [M^+] (20), 292 (6), 185 (8), 163 (7), 137 (8), 136 (7), 124 (15), 123 (100), 107 (11). ^1H and ^{13}C NMR data, see Table 1.

Acetylation of 1. To a solution of pyridine (0.5 mL) and acetic anhydride (0.5 mL) was added compound **1** (7.0 mg) and the mixture allowed to stand at room temperature for 24 h. Cold H_2O was added, and the diacetyl derivative (7.6 mg) was extracted with $CHCl_3$: CIMS (methane) *m/z* 431 [M + 1]$^+$ (55), 405 (48), 389 (33), 377 (100), 375 (13), 361 (13), 33 (11), 333 (14); ^1H NMR (300 MHz, $CDCl_3$) δ 7.03 (d, J = 8.6 Hz, H-6), 6.95 (dd, J = 2.7, 8.6 Hz, H-5), 6.92 (d, J = 2.7 Hz, H-3), 5.35 (t, J = 4.8 Hz, H-10′ and H-11′), 2.48 (t, J = 8.0 Hz, H-1′), 2.32 and 2.29 (s, 2 × $OCOCH_3$), 0.89 (t, J = 5.8 Hz).

2-[10′,11′-Bis(methylthio)heptadecanyl]-1,4-hydroquinone. Dimethyl disulfide (1 mL) and the diacetyl derivative of **1** (7 mg) were mixed with a catalytic trace of iodine, and the mixture was stirred for 24 h at room temperature under nitrogen atmosphere. Et_2O was added, and the solution was washed with KOH aqueous solution. The Et_2O phase was evaporated to afford the dimethyl sulfide derivative (7.5 mg): EIMS *m/z* 524 [M^+, $C_{25}H_{44}O_2S_2$], 477 (5), 379 (100), 337 (20), 295 (8), 165 (28), 145 (30) and 123 (92).

(4*R*,6*R*)-Dihydroxy-4-[10(*Z*)-heptadecenyl]-2-cyclohexenone (2): oil, $[\alpha]_D$ +45.5° (*c* 0.004, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 nm (3.46), 338 (1.95); IR (film) ν_{max} 3394, 2954, 2858, 1680, 1440, 1041; FABMS *m/z* 363 [M − 1]$^+$ (100), 344 (40), 311 (15), 279 (14), 125 (15), 100 (20); ^1H and ^{13}C NMR data, see Table 1.

(4*R*,6*S*)-Dihydroxy-4-[10′,11′-bis(methylthio)heptadecenyl]-2-cyclohexenone. A 5 mg portion of **2** was submitted to reaction with dimethyl disulfide (1 mL) using the procedure previously described. It afforded 5.6 mg of dimethyl sulfide derivative. FABMS *m/z* 458 [M^+] (10), 311 (12), 313 (63), 279 (16), 145 (100), 123 (18).

4-Chlorobenzoylation of 2. A solution of **2** (5.0 mg) and 4-chlorobenzoyl chloride (1 mL) was heated at 70 °C for 30 min. The reaction mixture was washed with H_2O saturated with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated and subjected to column chromatography over Si gel eluted with petroleum ether to separate the excess 4-chlorobenzoyl chloride. Elution with $CHCl_3$:EtOAc (7:3) afforded 6-[(4-chlorobenzoyl)oxy]-4-hydroxy-4-[heptadec-10(*Z*)-enyl]cyclohexenone (**4**, 6.0 mg) as a wax: UV (EtOH) λ_{max} (log ϵ) 240 (4.68); CD (*c* 0.03 mmol, MeOH) $\Delta\epsilon$ (nm) +8.20 (243); CIMS (methane) *m/z* 505 (4), 503 [M + 1]$^+$ (7), 487 (12), 485 (27), 349 (15), 347(92), 345 (18), 331 (11), 329 (33), 327 (11), 265 (15), 159 (30), 157 (100), 141 (20), 139 (66), 123 (26), 113 (21); ^1H NMR (400 MHz, pyridine-d_5) δ 8.14 (d, J = 9.0 Hz, H-2″ and H-6″), 7.49 (d, J = 9.0 Hz, H-3″ and H-5″), 6.43 (dd, J = 10.8, 2.5 Hz, H-3), 6.20 (dd, J = 10.8, 2.0 Hz, H-2), 5.46 (t, J = 4.2 Hz, H-10′ and H-11′), 5.36 (ddd, J = 5.5, 4.4, 2.0 Hz, H-6); 2.30−2.60 (m, H_2-5), 1.00−1.30 [m, $(CH_2)_n$], 0.84 (t, J = 5.9 Hz, H_3-17′).

Cytotoxicity Assay Procedures. The MeOH extract, chromatographic fractions, and the pure isolated compounds (**1** and **2**) were tested against a panel of human cancer cell lines (Table 1) using established protocols.[9]

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