New Chromanone Acids with Antibacterial Activity from *Calophyllum brasiliense*

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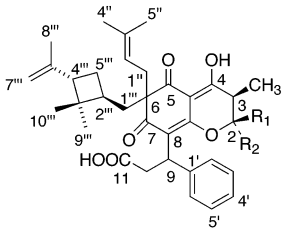
Received September 30, 2003

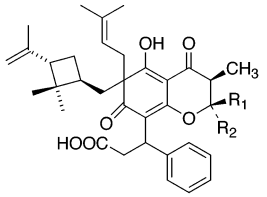
Six novel chromanone acids (**1**−**6**) were isolated from the bark of *Calophyllum brasiliense* Cambess. Their structures were elucidated on the basis of 1D and 2D NMR experiments, as well as mass spectrometry. All compounds showed moderate to strong antibacterial activity against *Bacillus cereus* and *Staphylococcus epidermidis*, with **1** and **2** being most active. None of the compounds were cytotoxic against KB, Jurkat T, and myosarcoma cancer cells up to 20 μg/mL.

As a part of our continuing search for bioactive compounds from plants employed in the traditional medicine of Mexico, *Calophyllum brasiliense* Cambess. (Clusiaceae), a large tree that grows in tropical forests from Mexico to Brazil, was selected for phytochemical investigation. Its bark latex is used by the Popoluca, an ethnic group living in a mountain range in southern Veracruz (Mexico), to treat toothache and to prevent wound infections by microorganisms.[1] In Brazil, the infusion and the bath prepared from the stem bark is a popular remedy for the treatment of rheumatism, varicose veins, hemorrhoids, and chronic ulcers.[2] The genus *Calophyllum* is known as a rich and valuable source of bioactive xanthones and coumarins especially since the isolation of the calanolides, prototypes of a unique subclass within the nonucleoside HIV-1 reverse transcriptase inhibitors, have been reported.[3−6] Phytochemical and biological investigation of the stem bark of *C. brasiliense* revealed the presence of several 4-substituted coumarins as well as xanthones.[7,8] Bioactivity-guided fractionation of the *n*-hexane and of the ethyl acetate extracts of the bark of *C. brasiliense* afforded six novel chromanone acids (**1**−**6**), four of them exhibiting an unusual cyclobutyl ring, with antibacterial activity against *Bacillus cereus* and *Staphylococcus epidermidis*. Although other chromanone acids have been isolated from the genus *Calophyllum*,[9−13] no biological studies on these compounds have been reported to our knowledge.



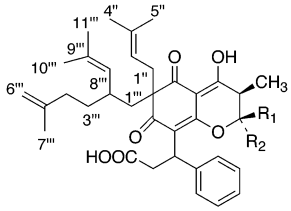
1: R_1 = H, R_2 = CH_3

2: R_1 = CH_3, R_2 = H



3: R_1 = H, R_2 = CH_3

4: R_1 = CH_3, R_2 = H



5: R_1 = H, R_2 = CH_3

6: R_1 = CH_3, R_2 = H

Results and Discussion

The dried and pulverized bark of *C. brasiliense* was extracted successively with *n*-hexane, ethyl acetate, and methanol. The *n*-hexane and ethyl acetate extracts showed antibacterial activity against *B. cereus* and *S. epidermidis* and were therefore subjected to further fractionation by vacuum-liquid chromatography (VLC), open column chromatography (CC) on silica gel, and HPLC, yielding six novel chromanone acids (**1**−**6**).

The ^{13}C NMR spectrum of **1** showed signals corresponding to 35 carbons, which were sorted by DEPT experiments into seven methyl, five methylene, 11 methine, and 12 quaternary carbons. The LRDEIMS of **1** gave a M^+ peak at *m*/*z* 560, which allowed, in combination with the NMR data, the establishment of the molecular formula $C_{35}H_{44}O_6$. Accordingly, a molecular ion peak was observed in the HRDEIMS at *m*/*z* 560.3132. ^{1}H and ^{13}C NMR data (Tables 1 and 2) pointed to the presence of a prenylated chromanone acid substituted with a 2-carboxyl-1-phenylethyl group. Proton signals corresponding to two methine (δ_H 2.62 dq, J = 6.3, 12.2 Hz; 4.10 dq, J = 6.5, 12.2 Hz) and two secondary methyl groups (δ_H 1.27 d, J = 6.3 Hz; 1.47 d, J = 6.5 Hz) confirmed the presence of the highly substituted 2,3-dimethylchromanone ring. A downfield shifted proton signal resonating at δ 16.63 (s) revealed the presence of a hydroxyl proton participating in a strong

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10.1021/np030438n CCC: $27.50
Published on Web 02/26/2004

Table 1. ^{1}H NMR Data for Compounds **1**−**6** (in $CDCl_3$, 295 K, δ ppm; J in Hz)

H	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
2	4.10 dq (6.5, 12.2)	4.45[c] m	4.23 dq (6.9, 12.5)	4.61 dq (4.0, 7.1)	4.12 dq (6.8, 12.7)	4.26[c] m
3	2.62 dq (6.3, 12.2)	2.70 dq (3.5, 7.2)	2.45 dq (6.9, 12.5)	2.54 dq (4.0, 7.1)	2.45 dq (7.1, 12.7)	2.56 dq (3.4, 7.5)
4-OH	16.63 s	16.34 s			16.45 s	16.22 s
5-OH			12.27 s	12.20 s		
9	4.84 m	4.84 m	4.80[c] m	4.80[c] m	4.90[c] m	4.90[c] m
10	3.20 dd (2.6, 7.4)	3.22 m, 2H	3.26 m, 2H	3.29 m, 2H	3.23 dd (2.0, 7.2)	3.28[c] m, 2H
	3.26 dd (2.0, 7.4)				3.32 dd (2.0, 7.2)	
2′, 6′	7.34 m	7.34 m	7.41 m	7.41 m	7.36[c] m	7.36[c] m
3′, 5′	7.12 m	7.12 m	7.11 m	7.11 m	7.23 m	7.23 m
4′	7.21 m	7.21 m	7.20 m	7.20 m	7.36[c] m	7.36[c] m
1″	2.47[c] m, 2H	2.54[c] m, 2H	2.46[c] m, 2H	2.64[c] m, 2H	2.39[c] m	2.33[c] m
					2.43[c] m	2.45[c] m
2″	4.54 m	4.54[c] m	4.50 m	4.50[c] m	4.50 m	4.50 m
4″	1.40 s	1.42 s	1.41 s	1.43 s	1.66 s	1.66 s
5″	1.39 s	1.34 s	1.44 s	1.46 s	1.34 s	1.30 s
1‴	1.99 dd (2.8, 13.6)	2.05 m	2.46[c] m	2.46[c] m	2.02 dd (2.9, 13.4)	1.99 dd (3.1, 13.3)
	2.18 dd (2.8, 13.6)	2.22 m	2.51[c] m	2.51[c] m	2.16 dd (2.9, 13.4)	2.12 dd (3.1, 13.3)
2‴	1.38[c] m	1.38[c] m	1.36[c] m	1.36[c] m	1.92[c] m	1.97[c] m
3‴					1.30[c] m	1.30[c] m
					1.32[c] m	1.32[c] m
4‴	2.33[c] m	2.38[c] m	2.35[c] m	2.35[c] m	1.76[c] m, 2H	1.76[c] m, 2H
5‴	1.45[c] m	1.45[c] m	1.42[c] m	1.42[c] m		
	1.66[c] m	1.69 m	1.62[c] m	1.65[c] m		
6‴					4.61 s	4.61 s
					4.66 s	4.66 s
7‴	4.38 s	4.45 s	4.40 s	4.42 s	1.66 s	1.64 s
	4.71 s	4.71 s	4.73 s	4.73 s		
8‴	1.59 s	1.57 s	1.57 s	1.59 s	4.90[c] m	4.90[c] m
9‴	0.75 s	0.73 s	0.75 s	0.72 s		
10‴	1.03 s	1.01 s	1.02 s	1.00 s	1.66 s	1.68 s
11‴					1.55 s	1.52 s
2-CH_3	1.47 d (6.5)	1.36 d (7.1)	1.51 d (6.9)	1.42 d (7.1)	1.44 d (6.8)	1.48 d (7.2)
3-CH_3	1.27 d (6.3)	1.19 d (7.2)	1.19 d (6.9)	1.09 d (7.1)	1.22 d (7.1)	1.18 d (7.5)

[a] Spectra recorded at 600.13 MHz. [b] Spectra recorded at 500.13 MHz. [c] Signals overlapped.

hydrogen bond. Two signals resonating at δ_C 196.0 (s) and 199.1 (s) indicated two unsaturated ketone carbonyl groups, and a signal resonating at δ_C 190.7 (s) pointed to the presence of an enol group.[12] Five aromatic protons were also evident in the ^{1}H NMR spectrum (Table 1) and indicated a monosubstituted phenyl moiety. The ^{1}H NMR and COSY spectra revealed the presence of a vinyl proton (δ_H 4.54, m) coupled to two vinylic methyl groups (δ_H 1.39 s, 1.40 s), indicating an isopent-2-enyl group. Two signals of terminal methylenes (δ_H 4.38 br s, 4.71 br s) together with a methyl group (δ_H 1.59 s) pointed to the presence of an isopropenyl group. HMBC correlations between H_2-1″ and C-5, C-6, and C-7 established that the isopent-2-enyl group was substituted at C-6 of the chromanone. HMBC correlations of the hydroxyl proton (δ_H 16.63 s) to C-3, C-4, C-4a, C-5, and C-6 determined the position of the hydroxyl group at C-4. Salient TOCSY cross-peaks (H_2-1‴/H-4‴, H-2‴/H-4‴, and H-2‴/H_2-5‴) and HMBC correlations between H_2-7‴ and C-4‴/C-8‴, H-4‴ and C-3‴/C-5‴/C-6‴/C-7‴/C-9‴/C-10‴, and H_2-1‴ and C-6/C-1″/C-2‴/C-3‴/C-5‴ supported the presence of a 3-isopropenyl-2,2-dimethylcyclobutylmethyl chain at C-6. Finally, HMBC cross-peaks between H-9 and C-7/C-8/C-10 and H_2-10 and C-7/C-8/C-11 clearly showed that the 2-carboxyl-1-phenylethyl moiety must be substituted at C-8 of the chromanone. DQF-COSY, TOCSY, HSQC, HSQC-TOCSY, HMBC, and ROESY experiments allowed the complete assignment of all signals and the identification of compound **1** as 3-[4-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid. Comparison of the shift values of C-2, C-3 (**1**, δ_C 77.4 d, 42.6 d, respectively), and the attached methyl groups (δ_C 19.3, 11.6, respectively) as well as the signal pattern of H-2 and H-3 (Table 1) with literature data of 2,3-dimethylchro-

Table 2. ^{13}C NMR Data of Compounds **1**−**6** (in $CDCl_3$, 295 K, δ ppm, multiplicities were obtained from DEPT 135/DEPT 90 experiments)

C	**1**	**2**	**3**	**4**	**5**	**6**
2	77.4 d	74.5 d	81.0 d	78.8 d	77.2 d	74.4 d
3	42.6 d	40.5 d	44.4 d	43.3 d	41.6 d	39.7 d
4	190.7 s	191.3 s	196.9 s	199.1 s	187.6 s	188.2 s
4a	102.9 s	102.7 s	104.8 s	104.8 s	103.1 s	103.2 s
5	199.1 s	199.6 s	185.3 s	185.4 s	200.2 s	200.8 s
6	59.7 s	59.9 s	57.0 s	57.1 s	60.0 s	59.8 s
7	196.0 s	195.9 s	195.2 s	194.8 s	196.1 s	195.9 s
8	114.4 s	114.5 s	113.1 s	113.3 s	114.0 s	115.3 s
8a	163.7 s	163.0 s	166.1 s	166.0 s	163.8 s	167.7 s
9	35.6 d	35.2 d	35.1 d	34.9 d	35.4 d	35.2 d
10	36.7 t	36.2 t	36.3 t	36.1 t	36.3 t	36.0 t
11	177.9 s	178.1 s	177.0 s	176.8 s	177.5 s	177.7 s
1′	142.9 s	142.8 s	143.1 s	143.0 s	143.1 s	143.0 s
2′, 6′	127.7 d	127.7 d	127.8 d	127.8 d	127.6 d	127.6 d
3′, 5′	127.9 d	127.9 d	128.1 d	128.1 d	127.9 d	127.9 d
4′	125.9 d	125.9 d	125.9 d	125.9 d	125.8 d	125.8 d
1″	41.0 t	40.6 t	41.0 t	40.4 s	42.6 t	43.1 t
2″	117.3 d	117.2 d	117.6 d	117.0 d	116.8 d	116.7 d
3″	135.1 s	135.0 s	135.7 s	135.6 s	135.3 s	134.9 s
4″	25.6 q	25.5 q	25.6 q	25.5 q	25.5 q	25.6 q
5″	17.5 q	17.4 q	17.9 q	17.7 q	17.5 q	17.4 q
1‴	39.8 t	38.8 t	40.8 t	39.9 t	40.9 t	40.7 t
2‴	37.9 d	37.7 d	38.3 d	37.6 d	44.9 d	44.5 d
3‴	40.3 s	40.3 s	40.1 s	40.1 s	31.8 t	31.7 t
4‴	48.3 d	48.2 d	48.4 d	48.2 d	35.1 t	35.0 t
5‴	25.7 t	26.0 t	25.9 t	26.0 t	145.9 s	146.5 s
6‴	145.9 s	145.8 s	145.9 s	145.5 s	109.7 t	110.2 t
7‴	108.7 t	108.7 t	109.0 t	109.0 t	22.5 q	22.3 q
8‴	23.7 q	23.6 q	23.7 q	23.6 q	122.2 d	122.4 d
9‴	23.9 q	23.9 q	24.2 q	24.2 q	132.0 s	131.9 s
10‴	24.7 q	24.7 q	24.7 q	24.7 q	25.7 q	25.6 q
11‴					17.9 q	17.4 q
2-CH_3	19.3 q	16.3 q	19.1 q	15.8 q	19.3 q	16.6 q
3-CH_3	11.6 q	9.8 q	10.2 q	9.1 q	12.7 q	9.9 q

Table 3. Antibacterial and Cytotoxic Activity of Compounds **1**−**6**

	minimum inhibition concentration (MIC) in broth (μg/mL)		IC_{50} (μg/mL)		
compound	*B. cereus* (ATCC 10702)	*S. epidermidis* (ATCC 12228)	KB cells (ATCC CCL 17)	Jurkat T cells (ATCC TIB 152)	myosarcoma cells
1	1	16	>20	>20	>20
2	1	16	>20	>20	>20
3	4	16	>20	>20	>20
4	4	16	>20	>20	>20
5	16	16	>20	>20	>20
6	16	16	>20	>20	>20
chloramphenicol	4	4			
podophyllotoxin			0.005		
helenalin				0.03	
aculeatin A					0.3

manones[11,12] clearly indicated that compound **1** is the *trans* (*rel*-2*R*,3*R*) isomer.

The MS and ^{1}H, ^{13}C, as well as the 2D NMR spectra of compound **2** were almost identical to those of compound **1**. The main differences were due to the shift values of C-2, C-3 (δ_C 74.5 d, 40.5 d, respectively) and the methyl groups at C-2 and C-3 (δ_C 16.3, 9.8, respectively) as well as the signal pattern of H-2 and H-3. Thus, compound **2** is the *cis* (*rel*-2*S*,3*R*) isomer of 3-[4-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**2**). We propose the trivial names brasiliensophyllic acid A (**1**) and isobrasiliensophyllic acid A (**2**), respectively.

The LRESIMS of compound **3** showed a pseudomolecular peak at *m*/*z* 583 $[M + Na]^+$, which corresponds, together with the obtained ^{13}C NMR data, to the molecular formula of $C_{35}H_{44}O_6$. Accordingly, a molecular ion could be observed at *m*/*z* 560.3129 in the HRDEIMS. The ^{1}H and ^{13}C NMR data obtained for **1** and **3** are almost identical (Tables 1 and 2), and the main differences are due to the chemical shifts of the hydroxyl proton (δ_H 12.27 s, **3**), the quaternary carbon C-4 (δ_C 196.9 s, **3**), and the carbons C-5 and C-6 (δ_C 185.3 s, 57.0 s, **3**). This information established the presence of a hydroxyl group at position 5, which was confirmed by HMBC correlations between the hydroxyl proton and C-4/C-6/C-1′. The comparison of the coupling constants of protons H-2 and H-3 of **1** and **3** indicated that compound **3** was also the *trans* (*rel*-2*R*,3*R*) isomer. Compound **4** was very similar to compound **3**, and application of the same structure elucidation strategy revealed that **4** is the *cis* (*rel*-2*S*,3*R*) form. The structures of compounds **3** and **4** were finally confirmed by DQF-COSY, TOCSY, HSQC, HSQC-TOCSY, HMBC, and ROESY experiments as 3-[*rel*-(2*R*,3*R*)-5-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-4,7-dioxo-3,4,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**3**) and 3-[*rel*-(2*S*,3*R*)-5-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-4,7-dioxo-3,4,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**4**). We propose the trivial names brasiliensophyllic acid B (**3**) and isobrasiliensophyllic acid B (**4**), respectively.

The LRDEIMS of **5** showed a M^+ peak at *m*/*z* 574, which, together with the ^{13}C NMR data (Table 2), corresponds to the molecular formula $C_{36}H_{46}O_6$. Comparison of 1D and 2D NMR data of **5** to those of **1** revealed only differences regarding the substituents at C-6. DQF-COSY and HMBC experiments suggested the presence of one 5-methyl-2-(2-methylpropenyl)hex-5-enyl chain linked to C-6. According to our NMR and MS analysis, we propose the structure 3-{*rel*-(2*R*,3*R*)-4-hydroxy-2,3-dimethyl-6-(3-methylbut-2-enyl)-6-[5-methyl-2-(2-methylpropenyl)hex-5-enyl]-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl}-3-phenylpropionic acid for compound **5**. NMR and MS data revealed that compound **6** is 3-{*rel*-(2*S*,3*R*)-4-hydroxy-2,3-dimethyl-6-(3-methylbut-2-enyl)-6-[5-methyl-2-(2-methylpropenyl)hex-5-enyl]-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl}-3-phenylpropionic acid and thus the corresponding *trans* isomer. We propose the trivial names brasiliensophyllic acid C (**5**) and isobrasiliensophyllic acid C (**6**), respectively.

All new compounds showed moderate to strong antibacterial activity against the Gram-positive bacteria *B. cereus* and *S. epidermidis* (Table 3). Compounds **1** and **2** were even more active than the reference compound chloramphenicol against *B. cereus*, while compounds **5** and **6** are less active. Thus, the presence of a cyclobutyl ring in compounds **1**−**4** most probably contributes to the strong antibacterial activity of the isolated chromanone acids against this organism. None of the compounds were cytotoxic against KB (HeLa), Jurkat T, and myosarcoma cancer cell lines (IC_{50} >20 μg/mL). The antibacterial activities of these metabolites support the traditional use of *C. brasiliense* in Mexico for wound healing.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter using MeOH as solvent. UV spectra were obtained in MeOH on a UVIKON 930 spectrometer. ^{13}C NMR spectra were measured on a Bruker AMX-300 (operating at 300.13 MHz for ^{1}H and 75.47 for ^{13}C). ^{1}H and 2D spectra of **1** and **2** were recorded on a Bruker DRX-600 spectrometer (operating at 600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C). All other spectra were measured on a Bruker DRX-500 spectrometer (operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C). All spectra were measured at 295 K in $CDCl_3$ and referenced against residual $CHCl_3$ in $CDCl_3$ (^{1}H 7.27 ppm) and $CDCl_3$ (^{13}C 77.0 ppm). LRDEIMS and HRDEIMS were measured on a micromass TRIBRID double-focusing mass spectrometer at 70 eV. ESIMS were measured on a TSQ 7000. HPLC separations were performed with a Merck-Hitachi L-7150 pump, connected to a Merck-Hitachi UV L-7400 detector, a Merck-Hitachi L-7200 autosampler, a Merck-Hitachi D 7000 interface, and a Varian HPLC column (Microsorb 300 C-4, 10 μm, 250 × 4.6 mm) or a Knauer HPLC column (Eurospher 80 C-2, 10 μm, 250 × 4.6 mm). All solvents used were of HPLC grade.

Plant Material. The bark of *Calophyllum brasiliense* Cambess. (Clusiaceae) was collected in Soteapan (Mexico). The plant was collected and identified by Dr. M. Leonti (ETH Zurich, Switzerland), Dr. M. Sousa, A. Reyes, and F. Ramos (all Mexican National Herbarium, Mexu). A voucher specimen is deposited at the Mexu with the identification number L 267.

Extraction and Isolation. Air-dried and powdered bark of *C. brasiliense* (400 g) was percolated successively with

n-hexane, EtOAc, and MeOH to afford 20.3 g of *n*-hexane-soluble material and 18.5 g of EtOAc extract. The *n*-hexane extract (20.3 g) was subjected to VLC over silica gel (40−60 μm, 300 g) using a step gradient of *n*-hexane, *n*-hexane with increasing amounts of CH_2Cl_2 (25% each step), CH_2Cl_2, CH_2Cl_2 with increasing amounts of EtOAc (25% each step), EtOAc, EtOAc with increasing amounts of MeOH (25% each step), and MeOH (700 mL each step). The collected fractions were evaporated under vacuum and examined by TLC. Homogeneous fractions were pooled to give 16 major fractions (F1−F16). Fraction F9 (1.3 g) was subjected to open column chromatography on silica gel (130 g) using CH_2Cl_2−MeOH (29:1, 1600 mL; 19:1, 800 mL) as eluent. Thirteen subfractions (F9.1−F9.13) were obtained. The antibacterial active subfraction F9.7 (150 mg) was further fractionated by CC on silica gel (60 g) and eluted with a mixture of CH_2Cl_2−MeOH (29:1, 1200 mL) to yield a mixture of compounds **1** and **2** (19.6 mg). The mixture was separated by reversed-phase (RP-4) HPLC using acetonitrile−H_2O−trifluoracetic acid (80:19.5:0.5 → 90:9.75:0.25, 25 min, 0.6 mL/min) to yield pure **1** (12 mg; t_R 9 min) and **2** (7 mg; t_R 11 min). The EtOAc extract (18.5 g) was applied to VLC over silica gel (40−60 μm, 280 g). Elution with CH_2Cl_2 containing increasing amounts of EtOAc, and with EtOAc containing increasing amounts of MeOH (700 mL each step), yielded 45 fractions. On the basis of the TLC similarities, identical fractions were combined to give a total of 11 fractions (G1−G11). Fraction G3 (1 g) was subjected to open column chromatography on silica gel (130 g) using a mixture of *n*-hexane−EtOAc (75:25, 1200 mL) to give 24 subfractions (G3.1−G3.24). Subfractions G3.21 and G3.23 showed antibacterial activity. G3.21 (120 mg) was subjected to open column chromatography on silica gel (30 g) and eluted with a mixture of CH_2Cl_2−MeOH−H_2O (15:1:0.1, 400 mL) to give a mixture of compounds **3** and **4** (5.4 mg). The mixture was separated by reversed-phase (RP-2) HPLC using acetonitrile−H_2O−trifluoracetic acid (60:39.5:0.25 → 70:29.5:0.25, 30 min, 1.0−0.9 mL/min) to yield pure **3** (2 mg; t_R 9 min) and **4** (2 mg; t_R 10 min). The subfraction G3.23 (36.2 mg) was further fractionated by CC on silica gel (6 g) and eluted with a mixture of *n*-hexane−EtOAc (65:35, 400 mL) to furnish a mixture of compounds **5**/**6** (7.3 mg). These were separated by reversed-phase (RP-4) HPLC using acetonitrile−H_2O−trifluoracetic acid (70:29.5:0.25 → 80:19.5:0.25, 25 min, 0.7 mL/min) as eluent, yielding pure **5** (4 mg; t_R 13 min) and **6** (2 mg; t_R 14 min).

Antibacterial Activity. The test organisms were *Bacillus cereus* (ATCC 10702, Gram-positive) and *Staphylococcus epidermidis* (ATCC 12228, Gram-positive). Antibacterial assays were carried out by the doubling dilution method using a recently published protocol.[14] Chloramphenicol was used as a positive control.

Cytotoxic Activity. The test was carried out with KB (HeLa, ATCC CCL 17), Jurkat T helper (ATCC TIB 152), and myosarcoma cells following recently published protocols.[15−17] Podophyllotoxin, helenalin, and aculeatin A were used as positive controls.

Brasiliensophyllic acid A (1): 3-[*rel*-(2*R*,3*R*)-4-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**1**), green gum (12 mg); $[\alpha]^{20}_D$ −5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (2.0), 312 (0.82) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 560 $[M]^+$ (5), 545 (5), 492 (83), 423 (91), 29 (100); HRDEIMS *m*/*z* 560.3132 $[M]^+$ (calcd for $C_{35}H_{44}O_6$ 560.3138).

Isobrasiliensophyllic acid A (2): 3-[*rel*-(2*S*,3*R*)-4-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**2**); green gum (7 mg); $[\alpha]^{20}_D$ −12° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (2.0), 312 (0.82) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 560 $[M]^+$ (5), 545 (5), 492 (83), 423 (91), 29 (100); HRDEIMS *m*/*z* 560.3132 $[M]^+$ (calcd for $C_{35}H_{44}O_6$ 560.3138).

Brasiliensophyllic acid B (3): 3-[*rel*-(2*R*,3*R*)-5-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-4,7-dioxo-3,4,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**3**); yellow gum (2 mg); $[\alpha]^{20}_D$ −25° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (1.56), 296 (0.70) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 560 (7) $[M]^+$, 542 (1), 351 (100); ESIMS (positive mode) *m*/*z* 583 (100) $[M + Na]^+$; HRDEIMS *m*/*z* 560.3129 $[M]^+$ (calcd for $C_{35}H_{44}O_6$ 560.3138).

Isobrasiliensophyllic acid B (4): 3-[*rel*-(2*S*,3*R*)-5-hydroxy-6-(3α-isopropenyl-2,2-dimethylcyclobutyl-*β*-methyl)-2,3-dimethyl-6-(3-methylbut-2-enyl)-4,7-dioxo-3,4,6,7-tetrahydro-2*H*-chromen-8-yl]-3-phenylpropionic acid (**4**); yellow gum (2 mg); $[\alpha]^{20}_D$ −49° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (1.56), 296 (0.70) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 560 (7) $[M]^+$, 542 (1) 351 (100); ESIMS (positive mode) *m*/*z* 583 (100) $[M + Na]^+$; HRDEIMS *m*/*z* 560.3129 $[M]^+$ (calcd for $C_{35}H_{44}O_6$ 560.3138).

Brasiliensophyllic acid C (5): 3-{*rel*-(2*R*,3*R*)-4-hydroxy-2,3-dimethyl-6-(3-methylbut-2-enyl)-6-[5-methyl-2-(2-methylpropenyl)hex-5-enyl]-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl}-3-phenylpropionic acid (**5**); green gum (4 mg); $[\alpha]^{20}_D$ −30° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (1.70), 285 (0.56) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 575 $[M + H]^+$ (1), 560 (6), 423 (4), 406 (6), 369 (28), 351 (100), 309 (40); HRDEIMS *m*/*z* 574.3342 (calcd for $C_{36}H_{44}O_6$ 574.3347).

Isobrasiliensophyllic acid C (6): 3-{*rel*-(2*S*,3*R*)-4-hydroxy-2,3-dimethyl-6-(3-methylbut-2-enyl)-6-[5-methyl-2-(2-methylpropenyl)hex-5-enyl]-5,7-dioxo-3,5,6,7-tetrahydro-2*H*-chromen-8-yl}-3-phenylpropionic acid (**6**); $[\alpha]^{20}_D$ −56° (*c* 0.1, MeOH); green gum (2 mg); UV (MeOH) λ_{max} (log ϵ) 206 (1.70), 285 (0.56) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRDEIMS *m*/*z* 575 $[M + H]^+$ (1), 560 (6), 423 (4), 406 (6), 369 (28), 351 (100), 309 (40), 69 (29); HRDEIMS *m*/*z* 574.3342 (calcd for $C_{36}H_{44}O_6$ 574.3347).

Acknowledgment. Special thanks are given to Dr. M. Leonti (Institute of Pharmaceutical Sciences, ETH Zurich) for collection and identification, as well as Dr. M. Sousa, A. Reyes, and F. Ramos (all Mexu) for identification of the plant material. We are indebted to Dr. O. Zerbe (Institute of Pharmaceutical Sciences, ETH Zurich) for recording NMR spectra, and we thank Dr. W. Amrein, Mr. R. Häfliger, and Mr. O. Greter (Institute of Organic Chemistry, ETH Zurich) for recording mass spectra. We are indebted to Ms. B. Bosilij (Zentrum für Operative Medizin, Heinrich-Heine-University Dusseldorf) for performing the myosarcoma assay.

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NP030438N