Cytotoxic and Antiplasmodial Xanthones from *Pentadesma butyracea*

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Four new xanthones, butyraxanthones A−D (**1**−**4**), were isolated from the stem bark of *Pentadesma butyracea*, together with six known xanthones (**5**−**10**) and a triterpenoid (lupeol). The structures of **1**−**4** were established by spectroscopic methods. Compounds **1**−**10** were tested in vitro for antiplasmodial activity against a *Plasmodium falciparum* chloroquine-resistant strain and for cytotoxicity against a human breast cancer cell line (MCF-7). Nearly all of these xanthones exhibited good antiplasmodial activity, and some of them also demonstrated potent cytotoxicity.

Pentadesma butyracea Sabine (Clusiaceae), commonly called "butter tree", is a multipurpose rain forest species widely distributed in tropical West Africa including Cameroon.[1] Different parts of this plant have been used in several African countries in folk medicine for the treatment of various diseases. In Gabon and in the Ivory Coast, the bark decoction is used as a purgative.[1] The bark serves in Ghana as a fish poison. In Congo-Brazzaville, the bark is sometimes taken as an aphrodisiac.[2] Moreover, in Benin and in the western part of Cameroon, an infusion of the stem bark is used to treat fever.[2] A root decoction is used in Liberia as a vermifuge, while the leaves after roasting and crushing are given to children in Sierra Leone to relieve constipation.[2]

Medicinal plants of the Clusiaceae family are known to be sources of bioactive xanthones.[3−5] Previous phytochemical investigations of *P. butyracea* have reported the presence of prenylated xanthones and terpenoids.[6] As part of our continuing search for bioactive compounds from Cameroonian medicinal plants, we have examined the stem bark of *P. butyracea* and report herein on the isolation and structure elucidation of four new xanthones, namely, butyraxanthones A−D (**1**−**4**). All xanthones isolated (**1**−**10**) were evaluated for their antiplasmodial activity against the FcB1 *Plasmodium falciparum* chloroquine-resistant strain and for cytotoxicity against the MCF-7 human breast cancer cell line.

Compound **1** was obtained as a pale yellow powder and gave positive reaction with ferric chloride to indicate its phenolic nature. Its molecular formula, $C_{29}H_{34}O_6$, representing 13 unsaturations, was deduced from its HREIMS, showing the molecular ion peak at *m*/*z* 478.2357. The UV spectrum of compound **1** exhibited specific absorptions of a xanthone nucleus at λ_{max} 243, 315, and 442 nm.[7] The ^{1}H NMR spectrum of compound **1** (Table 1) gave signals of a hydrogen-bonded hydroxyl proton at δ_H 13.80 (s, OH-1) and two other free phenolic hydroxyls at δ_H 6.15 (s, OH-3) and 6.32 (s, OH-6). It also showed the signal of a methoxyl group at δ_H 3.80 (3H, s, OCH_3-7), the signals of two aromatic protons appearing as singlets at δ_H 6.29 (s, H-4) and 6.83 (s, H-5), and the characteristic signals of geranyl subunit protons at δ_H 3.46 (2H, d, J = 7.2 Hz, H-1a′), 5.29 (1H, t, J = 7.2 Hz, H-2′), 1.78 (3H, s, H-4′), 2.03 (2H, m, H-5′), 1.99 (2H, m, H-6′), 5.02 (1H, t, J = 6.4 Hz, H-7′), 1.55 (3H, s, H-9′), and 1.83 (3H, s, H-10′). Moreover, the ^{1}H NMR spectrum of compound **1** exhibited signals for a prenyl group in the *peri* position to the carbonyl function (C-8), according to its deshielded H-1″ resonance recorded at δ_H 4.09 (2H, d, J = 6.0 Hz, H-1″).[4] From additional NMR data of compound **1**, including the ^{13}C, DEPT, and HSQC spectra, the 29 carbon signals recorded (Table 1) could be grouped into one carbonyl carbon signal (δ_C 182.0, C-9), 12 carbons for two aromatic rings with six oxygenated carbons, one geranyl unit, one prenyl group, and one methoxyl carbon at δ_C 62.1. The heteronuclear multiple-bond correlation (HMBC) spectrum of compound **1** (Figure 1) showed long-range couplings between the hydrogen-bonded proton (OH-1) and C-1 (δ_C 160.7), C-2 (δ_C 108.7), and C-9a (δ_C 103.7). The correlations between the proton at δ_H 3.46 (H-1′) and C-1 (δ_C 160.7), C-2 (δ_C 108.7), and C-3 (δ_C 161.6) in the HMBC spectrum clearly demonstrated that the geranyl group is attached to C-2. The aromatic proton at δ_H 6.83 showed HMBC correlations to resonances at δ_C 112.4, 142.6, 154.5, and 155.1, while the hydroxyl group at δ_H 6.32 correlated with C-6 (δ_C 154.5) and C-5 (δ_C 101.5), and the methoxyl group at δ_H 3.80 showed a correlation with carbon C-7 (142.6), confirming the placement of the methoxyl and the hydroxyl group at C-7 and C-6, respectively. The attachment of the prenyl group to C-8 was deduced from the correlations observed in the same HMBC spectrum between the deshielded proton H-1″ (δ_H 4.09) of the prenyl group and carbons C-7 (δ_C 142.6), C-8 (δ_C 137.1), and C-8a (δ_C 112.4). Thus, compound **1** was deduced as 2-[3,7-dimethylocta-2,6-dien-1-yl]-1,3,6-trihydroxy-7-methoxy-8-(3-methylbut-2-en-1-yl)-9*H*-xanthen-9-one and has been named butyraxanthone A.

Compound **2** was isolated as a yellowish oil. Its HREIMS revealed a molecular formula of $C_{28}H_{30}O_6$. The UV spectrum showed specific absorptions of a xanthone nucleus at λ_{max} 204 and 281 nm.[7] The ^{1}H NMR spectrum of **2** exhibited signals of a hydrogen-bonded hydroxyl group at δ_H 13.76 and one aromatic proton at δ_H 6.29 (1H, s, H-4). Moreover, characteristic signals of two prenyl moieties were observed at δ_H 1.71 (3H, s, H-4″), 1.89 (3H, s, H-5″), 3.59 (2H, d, J = 7.3 Hz, H-1″), and 5.29 (1H, t, J = 7.3 Hz, H-2″) and δ_H 1.78 (3H, s, H-4‴), 1.88 (3H, s, H-5‴), 4.31 (2H, d, J = 6.9 Hz, H-1‴), and 5.30 (1H, t, J = 6.9 Hz, H-2‴). Characteristic signals of a 2,2-dimethylchromene ring were also observed in the ^{1}H NMR spectrum of compound **2** [δ_H 1.47 (6H, s, H-4′ and H-5′), 5.56 (1H, d, J = 10.1 Hz, H-2′), 6.73 (1H, d, J = 10.1 Hz, H-1′)].[8] The presence of this 2,2-dimethylchromene ring was confirmed by the set of carbon signals at δ_C 28.1 (C-

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Chart 1

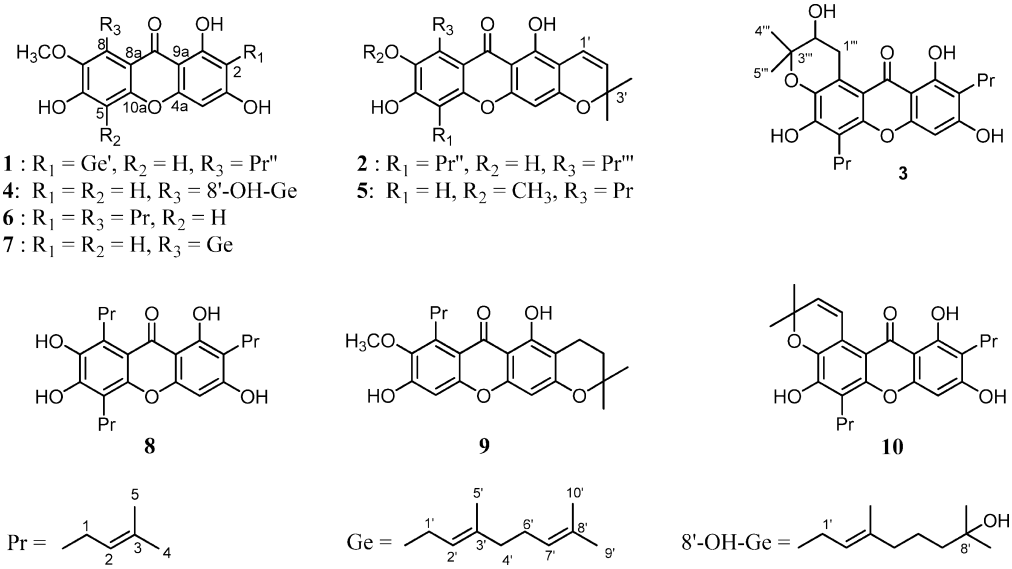

Table 1. NMR Data of Compounds **1** and **2** in $CDCl_3$ and **3** and **4** in CD_3OD

	butyraxanthone A (**1**)		butyraxanthone B (**2**)		butyraxanthone C (**3**)		butyraxanthone D (**4**)	
position	δ_H[a] (*J* in Hz)	δ_C, mult	δ_H[b] (*J* in Hz)	δ_C	δ_H[b] (*J* in Hz)	δ_C	δ_H[a] (*J* in Hz)	δ_C
1		160.7, qC		157.7, qC		154.6, qC		164.8, qC
2		108.7, CH		104.1, qC		113.2, qC	6.08, d (2.3)	98.8, CH
3		161.6, qC		159.5, qC		162.8, qC		166.0, qC
4	6.29, s	93.3, CH	6.29, s	93.8, CH	6.60, s	95.8, CH	6.17, d (2.3)	94.0, CH
4a		155.8, qC		156.1, qC		154.4, qC		158.1, qC
5	6.83, s	101.5, CH		113.3, qC		112.9, qC	6.69, s	102.9, CH
6		154.5, qC		151.1, qC		162.9, qC		158.4, qC
7		142.6, qC		139.2, qC		144.5, qC		144.9, qC
8		137.1, qC		124.4, qC		100.0, qC		138.6, qC
8a		112.4, qC		111.1, qC		105.2, qC		112.1, qC
9		182.0, qC		182.8, qC		174.2, qC		183.0, qC
9a		103.7, qC		103.8, qC		97.1, qC		103.9, qC
10a		155.1, qC		148.5, qC		154.1, qC		156.8, qC
1′	3.46, d (7.2)	21.5, CH_2	6.73, d (10.1)	115.6, CH	3.38, d (6.9)	21.9, CH_2	4.06, d (6.5)	27.0, CH_2
2′	5.29, t (7.2)	121.5, CH	5.56, d (10.1)	126.9, CH	5.25, t (6.9)	122.4, CH	5.22, t (6.5)	125.2, CH
3′		136.5, qC		77.7, qC		131.6, qC		135.6, qC
4′	1.99, m	39.7, CH_2	1.47, s	28.1, CH_3	1.78, s	25.6, CH_3	1.81, s	16.5, CH_3
5′	1.55, s	17.7, CH_3	1.47, s	28.1, CH_3	1.66, s	17.6, CH_3	1.97, d (6.4)	41.2, CH_2
6′	2.03, m	26.6, CH_2					1.36, m	44.2, CH_2
7′	5.02, t (6.4)	124.3, CH					1.43, m	23.5, CH_2
8′		135.6, qC						71.4, qC
9′	1.78, s	25.9, CH_3					1.10, s	29.1, CH_3
10′	1.83, s	16.5, CH_3					1.10, s	29.1, CH_3
1″	4.09, d (6.0)	26.5, CH_2	3.59, d (7.3)	22.5, CH_2	3.47, d (7.0)	22.3, CH_2		
2″	5.27, t (6.0)	123.3, CH	5.29, t (7.3)	120.6, CH	5.24, t (7.0)	122.5, CH		
3″		131.3, qC		133.4, qC		131.4, qC		
4″	1.60, s	25.6, CH_3	1.71, s	25.7, CH_3	1.65, s	25.7, CH_3		
5″	1.84, s	17.9, CH_3	1.89, s	17.8, CH_3	1.88, s	17.7, CH_3		
1‴			4.31, d (6.9)	25.8, CH_2	2.90, dd (16.4, 12.5)	22.0, CH_2		
					3.37, dd (16.4, 3.3)			
2‴			5.30, t (6.9)	121.7, CH	4.48, dd (12.5, 3.3)	87.5, CH		
3‴		135.2, qC				71.1, qC		
4‴			1.78, s	25.8, CH_3	1.48, s	26.3, CH_3		
5‴			1.88, s	17.8, CH_3	1.43, s	23.8, CH_3		
OCH_3-7	3.80, s	62.1, CH_3					3.77, s	61.4, CH_3
OH-1	13.80, s		13.76, s					
OH-3	6.32, s							
OH-6	6.15, s							

[a] Recorded at 300 MHz. [b] Recorded at 500 MHz.

4′,C-5′), 77.7 (C-3′), 115.6 (C-1′), and 126.9 (C-2′).[8] In the HMBC spectrum of **2**, the olefinic proton of the pyran group at δ_H 6.73 (H-1′) showed a cross-peak with carbons at δ_C 104.1 (C-2), 157.7 (C-1), and 159.5 (C-3), while in the NOESY spectrum, it showed an interaction with the proton of the chelated hydroxyl group at δ_H 13.76 (C-1-OH). This finding clearly indicated that the 2,2-dimethylchromene ring was fused in a linear way to the xanthone nucleus at positions C-2/C-3. In the same HMBC spectrum, correlations were observed between the deshielded methylene protons of the prenyl group at δ_H 4.31 (H-1‴) and carbons C-7 (δ_C 139.2) and C-8a (δ_C 111.1) to indicate its attachment to C-8. Furthermore, the C-5 position of the second prenyl group was deduced from the correlations between the protons of the methylene group at δ_H 3.59 (H-1″) and the carbons C-10a (δ_C 148.5) and C-6 (δ_C 151.1). The HMBC spectrum of compound **2** also showed correlations between the aromatic proton at δ_H 6.29 and C-2 (δ_C 105.1) and C-9a (δ_C 103.6) to indicate its location at C-4. According to its molecular formula, the xanthone nucleus of compound **2** was

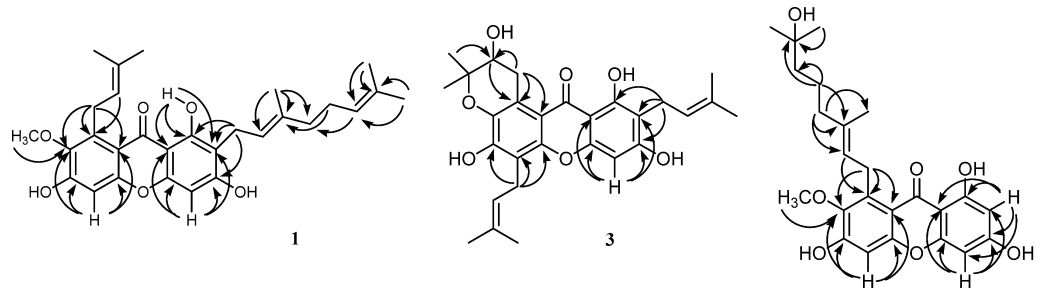

Figure 1. Key HMBC correlation of compounds **1**, **3**, and **4**.

finally substituted at the C-6 and C-7 by hydroxyl groups. This compound (butyraxanthone B) was therefore assigned as 5,8,9-trihydroxy-2,2-dimethyl-7,10-bis(3-methylbut-2-en-1-yl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one.

The molecular formula of compound **3** was established by HRCIMS (*m*/*z* 481.2229 ([M + H]$^+$) as $C_{28}H_{32}O_7$, requiring 13 degrees of unsaturation. The UV spectrum of compound **3** showed specific absorptions of the xanthone nucleus at λ_{max} 323, 427, and 453 nm.[7] The ^{13}C NMR spectrum of this compound displayed 28 carbon signals including six methyl groups, three saturated methylene groups, four methine carbons, and 15 unsaturated carbons including a carbonyl group at δ_C 174.2 and six aromatic carbon links to oxygen at δ_C 154.6 (C-1), 162.8 (C-3), 162.9 (C-6), 154.4 (C-4a), 154.1(C-10a), and 162.9 (C-7). The ^{1}H NMR spectrum of compound **3** revealed a singlet of one aromatic proton at δ_H 6.60. It also showed signals of two prenyl moieties with saturated methylene protons [δ_H 3.38 (2H, d, J = 6.9, H-1′) and 3.47 (2H, d, J = 7.0, H-1″)] and the characteristic signals of a 2-hydroxy-3,3-dimethylchroman moiety [δ_H 2.90 (dd, J = 16.4, 12.5, H-1a‴), 3.37 (dd, J = 16.4, 3.3, H-1b‴), 4.48 (dd, J = 12.5, 3.3, H-2‴), 1.78 (s, H-4‴), 1.66 (s, H-5‴)]. These data were very close to those reported for garcimangosone C, a xanthone isolated from *Garcinia mangostana*,[9] except for an additional prenyl group identified through the NMR data of compound **3**. The location of this last prenyl at C-5 of the xanthone nucleus was deduced from the HMBC spectrum, in which the protons of the methylene group at δ_H 3.47 (H-1″) showed correlations with carbons at δ_C 112.9 (C-5), 154.1 (C-10a), and 162.9 (C-6). The observation of long-range ^{1}H−^{13}C correlations between the saturated methylene protons of the chroman moiety (H-1‴) and the shielded aromatic carbons at δ_C 100.0 (C-8) and 105.2 (C-8a) supported the 7,8-fused position of the chroman ring. Further inspection of the HMBC NMR data of **3** finally allowed this compound to be identified as 2,5,9,11-tetrahydroxy-3,3-dimethyl-6,10-bis(3-methylbut-2-en-1-yl)-2,3-dihydropyrano[3,2-*a*]xanthen-12(1*H*)-one (butyraxanthone C).

Compound **4** (butyraxanthone D) was obtained as a red oil. Its HREIMS revealed a molecular formula of $C_{24}H_{28}O_7$. The UV spectrum of compound **4** showed specific absorptions of a xanthone nucleus at λ_{max} 243, 315, and 442 nm. The ^{1}H NMR spectrum of compound **4** showed one AB spin system at δ_H 6.08 (1H, d, J = 2.3 Hz, H-2) and 6.17 (1H, d, J = 2.3 Hz, H-4), characteristic of an 1,3-dihydroxybenzene moiety. It also exhibited the signals of a additional aromatic proton at δ_H 6.69 (1H, s, H-5), a methoxyl group at δ_H 3.77 (s, 3H), and the characteristic signals of protons of a geranyl substructure. The relative locations of these elements on ring B were then deduced by interlocking different HMBC correlations, namely, the correlations observed between the aromatic proton at δ_H 6.69 (H-5) and C-6 (δ_C 158.4), C-10a (δ_C 156.8), C-7 (δ_C 144.9), and C-8a (δ_C 112.1), as well as those evident between the methylene protons of the geranyl subsituent at δ_H 4.06 (2H, d, J = 6.5 Hz, H-1′) and C-8 (δ_C 138.6), C-7, and C-8a. Compound **4** was very similar to the known rubraxanthone (**7**), isolated from *Garcinia pyrifera,*[10] which differed from **4** by the loss of an H_2O unit according to their respective molecular formulas. Indeed, the only difference was observed on the geranyl chain, in which the second double bond was hydrated by comparison with the structure of rubraxanthone (**7**). This was confirmed by the presence of an oxygenated carbon in the ^{13}C NMR spectrum at δ_C 71.4 on one side and on the other side by the correlations observed in the HMBC spectrum of **4**. Correlations were observed between the proton H-10′ (δ_H 1.36) and C-7′ (δ_C 23.5) and C-8′ (δ_C 71.4) and between H-7′ (δ_H 1.43) and C-8′, C-9′, and C-10′ (δ_C 29.1). Thus, the structure of butyraxanthone D was concluded to be 3,6,8-trihydroxy-1-[(2*Z*)-7-hydroxy-3,7-dimethyloct-2-en-1-yl]-2-methoxy-9*H*-xanthen-9-one (**4**).

Table 2. Antiplasmodial Activity and Cytotoxicity of Compounds from *P. butyracea*

compound	antiplasmodial activity (IC_{50}, μg/mL)	MCF-7 (IC_{50}, μg/mL)	CAR[a]
1	3.0 ± 1.1	3.5 ± 0.6	1.17
2	2.7 ± 0.8	3.3 ± 0.6	1.22
4	>10	1.3 ± 0.5	<0.13
5	1.9 ± 0.5	3.9 ± 0.9	2.05
6	2.9 ± 1.0	3.5 ± 0.5	1.21
7	3.4 ± 0.5	2.6 ± 0.9	0.76
8	2.8 ± 0.2	1.5 ± 0.2	0.54
9	3.1 ± 0.9	1.2 ± 0.1	0.39
10	2.8 ± 1.2	2.6 ± 0.3	0.93
chloroquine	0.19	>100	>500
doxorubicin	ND	4.5	

[a] Cytotoxicity/antiplasmodial ratio.

In addition, six known compounds were isolated and identified by comparing their spectroscopic data with those of reported values, namely, mangostanin (**5**),[11] 1,3,6-trihydroxy-7-methoxy-2,8-diprenylxanthone (**6**),[12] rubraxanthone (**7**),[8] garcinone E (**8**),[13] gartanin (**9**),[14] tovophyllin (**10**),[15] and lupeol.[16]

Compounds **1**−**10** were tested in vitro for their antiplasmodial activity against the *Plasmodium falciparum* chloroquine-resistant FcB1 strain (Table 2). Among all tested compounds, only butyraxanthone D (**4**) was inactive (IC_{50} > 10 μg/mL). Other isolated compounds exhibited antiplasmodial activity against the FcB1 strain, with compound **5** showing the best potency. Xanthones are known for their antiplasmodial potency,[17,18] which may be due to an ability to inhibit heme polymerization, an important target of already available antimalarial drugs (e.g., chloroquine).[19] For compounds based on the xanthone skeletal feature it is difficult at this stage to define the contribution of the different functional groups with respect to activity, as all active compounds obtained in the present study had nearly the same potency. However, it is known that the presence of prenyl, geranyl, or hydroxyl groups at certain positions of the xanthone nucleus increases the resultant activity. A relevant observation is the absence of antiplasmodial activity in compound **4**, which could be obtained by the reduction of the active compound **7**. Xanthones **1**−**10** were also tested for cytotoxicity against the human breast cancer cell line MCF7 in vitro. All the tested compounds were cytotoxic, with compounds **4**, **8**, and **9** demonstrating the highest cytotoxicity.

Experimental Section

General Experimental Procedures. The optical rotation was measured on a Perkin-Elmer polarimeter. NMR spectra were recorded

on Bruker DRX 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) and DRX 300 (300 MHz for ^{1}H) instruments. Chemical shifts were reported with TMS as internal standard. Mass spectra (EI and CI) were recorded with a GC/MS Nermag R10-10 mass spectrometer. HRCI/MS were recorded with a Thermo Finnigan Mat 95XL mass spectrometer. TLC was carried out using Merck silica gel Si 60 F_{254} 20 × 20 cm aluminum sheets and RP-18 $F_{254}S$ 20 × 20 cm aluminum sheets. Analytical HPLC was carried out on a Thermo Separation Products system equipped with a P-4000 quaternary gradient pump, a UV-6000LP photodiode array detector, using analytical 125−4 mm columns packed with Merck Lichrospher 100 RP-18 (5 μm), and Macherey-Nagel Nucleosil 100-5 C_6H_6 (end capped). HPLC purifications were performed with a gradient solvent system (water−acetonitrile) and a flow rate of 1 mL/min. This yielded products with chemical purity greater than 93%. Medium-pressure liquid chromatography was carried out using Merck silica gel 60 (40−63 μm) or Lichroprep 60 RP-18 (40−63 μm) with UV detection at 254 and 366 nm.

Plant Material. The stem bark of *Pentadesma butyracea* was collected in June 2004 at Bazou in the West Province of Cameroon. The plant was identified by Mr. Nana Victor, botanist at the National Herbarium of Cameroon, where a voucher specimen (6861/SRF/Cam) has been deposited.

Extraction and Isolation. The dried and powdered stem bark (3 kg) of *Pentadesma butyracea* was extracted twice with methanol at room temperature, and the combined extract concentrated to dryness to obtain a viscous residue (200 g). A 5 g aliquot of the methanol crude extract was subjected to MPLC (RP-18, 460 × 26 mm) using H_2O−MeOH mixtures as mobile phase (flow rate 10 mL/min). Altogether, 250 fractions of 50 mL each were collected and grouped on the basis of the TLC analysis to yield 15 main fractions, A to O. From fraction D (124 mg), compound **9** (12 mg) was obtained after column chromatography on silica gel eluted with CH_2Cl_2 followed by crystallization from *n*-hexane−CH_2Cl_2 (1:1). Column chromatography of F (823 mg) over silica gel eluting with CH_2Cl_2 afforded compounds **6** (25 mg) and **7** (18 mg). Fraction G (45 mg) was subjected to column chromatography on Sephadex LH-20 and eluted with methanol to afford compounds **3** (5 mg) and **8** (10 mg). Fraction J (200 mg) was chromatographed over silica gel (70−230 mesh), eluting with a mixtures of *n*-hexane and methylene chloride of increasing polarity, to yield a mixture purified by passage over Sephadex LH-20, using methanol as eluent, to afford compounds **4** (10 mg) and **5** (20 mg). Compounds **1** (10 mg) and **2** (5 mg) were obtained from fraction M (105 mg) after column chromatography on silica gel, using CH_2Cl_2 as eluent, while lupeol (100 mg) was obtained from fraction O (220 mg).

Butyraxanthone A (1): yellow powder (*n*-hexane); mp 131−133 °C; UV (MeOH) λ_{max} (log ϵ) 243 (2.90), 315 (2.70), 442 (1.82) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 478 $[M]^+$, 409, 353; HREIMS *m*/*z* 478.2357 $[M]^+$ (calcd for $C_{29}H_{34}O_6$ 478.2355).

Butyraxanthone B (2): pale yellow oil ($CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (2.89), 281 (2.86) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 463 $[M + H]^+$, 409, 407; HREIMS *m*/*z* 462.2040 $[M]^+$ (calcd for $C_{28}H_{30}O_6$ 462.2042).

Butyraxanthone C (3): pale yellow oil (MeOH); $[\alpha]_D^{25}$ −24.4 (*c* 0.09, acetone); UV (MeOH) λ_{max} (log ϵ) 323 (1.94), 427 (2.80), 453 (2.74) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 480 $[M]^+$, 479, 959, 995; HRCIMS *m*/*z* 481.2229 $[M + H]^+$ (calcd for $C_{28}H_{33}O_6$ 481.2226).

Butyraxanthone D (4): red oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (2.83), 253 (2.79), 348 (2.85) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 427 $[M - H]^+$; HREIMS *m*/*z* 428.1839 $[M]^+$ (calcd for $C_{24}H_{28}O_7$. 428.1835).

Assays on *Plasmodium falciparum* in Vitro. The FcB1-Columbia strains of *P. falciparum*, both being chloroquine-resistant [IC_{50} for chloroquine (Sigma; Saint-Quentin Fallavier, France): 186 nM], were cultured continuously according to Trager and Jensen et al.[20] Parasite cultures were synchronized by D-sorbitol lysis (5% of D-sorbitol in sterile water, D-sorbitol, Merck) as reported by Lambros and Vanderberg.[21] The antimalarial activities of purified compounds were evaluated by the radioactive micromethod described by Desjardins et al.,[22] with modifications reported by Benoit et al.[23] Parasite culture was incubated with each compound for 48 h. Parasite growth was estimated by [^{3}H]-hypoxanthine (Perkin-Elmer, Courtaboeuf, France) incorporation, with [^{3}H]-hypoxanthine being added to the plates 24 h before freezing. After a 48 h incubation, plates were frozen and defrosted, and each well was harvested on a glass fiber filter. Incorporated [^{3}H]-hypoxanthine was then determined with a beta-counter (1450-Microbeta Trillux, Wallac, Perkin-Elmer). IC_{50} values were determined by linear least-squares regression analysis.[24] The positive control was chloroquine.

Cytotoxicity Evaluation. Cytotoxicity of the pure compounds was estimated on human breast cancer cells (MCF-7). The cells were cultured in the same conditions as those used for *P. falciparum*, except for 5% human serum, which was replaced by 5% fetal calf serum (Cambrex, Verviers, Belgium). For the determination of compound cytotoxicity, cells were distributed in 96-well plates at 2 × 10^4 cells/well in 100 μL. Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after 24 and 72 h incubation, exactly as for the *P. falciparum* assay.[24] The positive control was doxorubicin.

References and Notes

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