New Prenylated Anthraquinones and Xanthones from *Vismia guineensis*

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From the roots of *Vismia guineensis* 23 structurally related compounds were isolated and identified. Ten of them are new constituents, namely 3-*O*-(2-hydroxy-3-methylbut-3-enyl)-emodin (**1**); 3-*O*-(2-methoxy-3-methylbut-3-enyl)-emodin (**2**); 1,8-dihydroxy-3-(2-methoxy-3-methylbut-3-enyloxy)-6-methylxanthone (**3**); 1,8-dihydroxy-3-geranyloxy-6-methylxanthone (**4**); 1,8-dihydroxy-3-isoprenyloxy-6-methylxanthone (**5**); 1,8-dihydroxy-3-(3,7-dimethyl-7-methoxyoct-2-enyloxy)-6-methylxanthone (**6**); 3-*O*-(*E*-3-hydroxymethylbut-2-enyl)-emodin (**7**); 3-*O*-(3-hydroxymethyl-4-hydroxybut-2-enyl)-emodin (**8**); 1,8-dihydroxy-3-(*E*-3-hydroxymethylbut-2-enyloxy)-6-methylxanthone (**9**); and 1,8-dihydroxy-3-(3-hydroxymethyl-4-hydroxybut-2-enyloxy)-6-methylxanthone (**10**). Their structures were established by means of EIMS and a combination of homonuclear and heteronuclear 2D NMR techniques. Furthermore, an *in vitro* preliminary screening of antimitotic activity of all the isolated compounds was also evaluated.

In the course of phytochemical studies of medicinal plants from Mali (Africa), we have investigated *Vismia guineensis* (L.) Choisy (Hypericaceae), a shrub locally called "Karidjakouma". This species is typical of tropical West Africa, and its bark and roots are employed in decoctions for internal and external usages in many skin diseases, such as dermatitis, leprosy, syphilis, herpes, scabies, and eczemas.[1] In Mali the plant is marketed as vaseline or karité butter ointments containing 1% "Karidjakouma" root for topical diseases.

The titled plant was locally identified as *Psorospermum guineense* Horch., but several synonyms are reported: *Psorospermum senegalense* Spach., *Hypericum guineense* L., *Vismia leonensis* Hook, *Vismia laurentii* De Wild., and *Vismia guineensis* (L.) Choisy. In particular, the last name seems to be the proper botanical terminology.[2]

In the literature a study of the essential oil (mainly α-pinene)[3] and an investigation of the root acetone extract of *V. guineensis* have been reported.[4] In the latter work 10 compounds were isolated and identified: *β*-sitosterol, 3-geranylemodin, 3-geranylemodin anthrone, madagascin anthrone, bianthrone A_1, vismione H, xanthone V_1, xanthone V_2, xanthone V_{1a}, and xanthone V_{2a}. The present paper deals with the isolation and identification from roots of *V. guineensis* collected in Mali of 23 compounds, including 10 new constituents, besides others previously isolated from the title plant or other *Vismia* and/or *Psorospermum* species.

All the quinonoids isolated were subjected to in vitro preliminary biological screening using cytogenetic assays in human peripheral blood lymphocytes. This experimental approach was used to provide information on either general toxicity (cytotoxic assay) and cell-cycle arrest ability (antimitotic assay) or mutagenic potential (genotoxic assay) of the compounds toward dividing lymphocytes.[5−9]

Results and Discussion

Dried and powdered roots were extracted in a Soxhlet apparatus with *n*-hexane, $CHCl_3$, and $CHCl_3$−MeOH (9:1). The residues obtained after evaporation of the solvents were fractionated by Sephadex LH-20 gel filtration using MeOH−$CHCl_3$ mixtures as eluents. Purification of the four fractions obtained from gel filtration of *n*-hexane extract yielded 11 constituents, including seven new derivatives. The known compounds were identified as 3-*O*-(3,7-dimethyl-6,7-dihydroxyoct-2-enyl)-emodin; 3-*O*-geranylemodin; madagascin; and 3-*O*-(3,7-dimethyl-7-hydroxyoct-2-enyl)-emodin.

From fraction I, by preparative TLC, compound **1** was isolated as a red-orange solid. The EIMS showed a $[M]^+$ peak at *m/z* 354 corresponding to the molecular formula $C_{20}H_{18}O_6$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 20 carbon signals that were sorted by DEPT experiments into Me × 2; OCH_2 × 1; OCH × 1; $=CH_2$ × 1; =CH × 4; =C × 9; C=O × 2 (Table 1). The IR spectrum showed bands at 3400 cm^{-1} due to OH functions and bands of a free carbonyl at 1685 and a chelated one at 1640 cm^{-1}. These data, together with those obtained from UV (absorptions at 408 and 450 nm) and ^1H NMR (two singlets, 1H each at *δ* 12.10 and 12.30 due to two OH *peri* to a carbonyl moiety), suggested the presence of a 9,10 dioxygenated anthraquinone.[10] Furthermore, in the ^1H NMR spectrum a singlet (3H) at *δ* 2.35 due to an aromatic methyl was also present with two AB spin systems (*J meta*) corresponding to four aromatic protons. Their *J* values led us to distinguish the first AB spin system at *δ* 6.70 and 7.29 ($J = 2.5$ Hz) and the other one at *δ* 6.98 and 7.65 ($J = 1.7$ Hz). NOESY experiments showed an interaction between the aromatic methyl and the AB system with $J < 2$. These data were in accordance with a 1,8-dihydroxy-6-methyl-9,10-anthraquinone derivative, and the *J* value and chemical shifts of the signals of the second AB spin system indicated that C-3 was substituted with an oxygen. Thus, this anthraquinone skeleton was identified as emodin.[11]

These suggestions were confirmed by the analysis of the ^{13}C NMR spectrum, which showed two carbonyl signals at 192.1 and 181.9 ppm (the chelated one and a nonchelated one), the characteristic aromatic resonances, and the aromatic methyl at 22.3 ppm (Table 1).[11]

The chain linked to carbon 3 was identified as 2-hydroxy-3-methylbut-3-enyloxy due to the presence of a broad

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Table 1. ^{13}C NMR Chemical Shift Assignments for Compounds **1**, **2**, **7**, **8**[a]

	compound			
carbon	**1**	**2**	**7**	**8**
C-1	165.5 (0)	165.4 (0)	165.7 (0)	165.7 (0)
C-1a	114.5 (0)	114.3 (0)	114.2 (0)	114.2 (0)
C-2	124.7 (1)	124.9 (1)	124.8 (1)	124.8 (1)
C-3	148.9 (0)	148.8 (0)	148.8 (0)	148.8 (0)
C-4	119.1 (1)	119.0 (1)	118.8 (1)	118.8 (1)
C-4a	136.0 (0)	136.0 (0)	136.0 (0)	136.0 (0)
C-5	108.0 (1)	108.2 (1)	108.1 (1)	108.1 (1)
C-5a	133.6 (0)	133.6 (0)	133.8 (0)	133.8 (0)
C-6	163.1 (0)	163.0 (0)	162.6 (0)	162.6 (0)
C-7	109.5 (1)	109.5 (1)	109.3 (1)	109.3 (1)
C-8	166.3 (0)	166.4 (0)	166.2 (0)	166.2 (0)
C-8a	111.0 (0)	111.2 (0)	111.0 (0)	111.0 (0)
C-9	192.1 (0)	192.0 (0)	192.0 (0)	192.0 (0)
C-10	181.9 (0)	182.0 (0)	181.6 (0)	181.6 (0)
C-11	22.3 (3)	22.5 (3)	22.4 (3)	22.4 (3)
C-12	73.5 (2)	72.8 (2)	65.7 (2)	66.0 (2)
C-13	74.2 (1)	76.0 (1)	121.6 (1)	121.6 (1)
C-14	145.7 (0)	144.6 (0)	140.0 (0)	142.0 (0)
C-15	112.9 (2)	113.1 (2)	68.5 (2)	66.1 (2)
C-16	19.0 (3)	19.3 (3)	15.0 (3)	55.9 (2)
OCH_3		58.8 (3)		

[a] Numbers in parentheses designate the number of attached protons at the respective carbon.

singlet (3H, δ 1.83) and two signals at δ 4.97 and 5.12 (1H each) that suggested the presence of the fragment $CH_2{=}C(CH_3)$, and signals at δ 4.05, 4.14, and 4.46 had chemical shifts, splitting, and *J* couplings in agreement with a $O{-}CH{-}CH_2{-}O$ portion.[12] This side chain was confirmed by the ^{13}C NMR analysis (Table 1).[12] Therefore, **1** is 3-*O*-(2-hydroxy-3-methylbut-3-enyl)-emodin, a new natural compound.

The residue obtained from fraction III was purified with Sephadex LH-20 to obtain seven new fractions, and by flash chromatography or preparative TLC pure compounds **2**−**7** were isolated. The EIMS of **2** showed a $[M]^+$ peak at *m/z* 368, corresponding to the molecular formula $C_{21}H_{20}O_6$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 21 carbon signals that were sorted by DEPT experiments into Me × 2; OCH_3 × 1; OCH_2 × 1; OCH × 1; $=CH_2$ × 1; =CH × 4; =C × 9; C=O × 2 (Table 1). The IR, UV, and NMR spectra showed a relationship between **1** and **2**. In fact, the anthraquinone skeleton of the two derivatives was the same, although the side chain in **2** was identified as 2-methoxy-3-methylbut-3-enyloxy. The etherification of the 2 position of the side chain was deduced by the absence of the OH signal (δ 5.60, 1H, s) in the proton spectrum and by the presence of a singlet (δ 3.95, 3H, s) due to a methoxyl moiety and was confirmed by the analysis of the ^{13}C NMR spectrum. Therefore, **2** was identified as 3-*O*-(2-methoxy-3-methylbut-3-enyl)-emodin, a new constituent.

Compounds **3**−**6** were structurally related and identified as xanthone derivatives. All showed the presence of a phenolic OH at C-1 and C-8 as evidenced by the presence of two singlets (1H) in the proton spectrum between δ 12 and 13 and confirmed by the ^{13}C NMR resonance at 184 ppm (double-chelated carbonyl). The absence of free OH at C-3 or C-6 was indicated by the absence in the UV spectrum of bathochromic shifts of the band at 330 nm, after addition of NaOAc,[11] and the presence of aromatic hydroxyls and a conjugated chelated ketone (1650 and 1590 cm^{-1}) in the IR spectrum. Evidence for the presence of a 1,8-dihydroxy-6-methyl-3-oxyxanthone moiety included a singlet (3H) at about δ 2.40 due to an aromatic methyl group and two AB spin systems with similar splittings, *J* couplings, and resonances; it was confirmed by data obtained from NOESY experiments and from ^{13}C NMR resonances. The side chains were identified by comparing the data with those of the corresponding anthraquinone derivatives.

Table 2. ^{13}C NMR Chemical Shift Assignments for Compounds **3**−**6**, **9**, **10**[a]

	compound					
carbon	**3**	**4**	**5**	**6**	**9**	**10**
C-1	166.8 (0)	166.4 (0)	166.2 (0)	165.9 (0)	166.6 (0)	166.3 (0)
C-1a	104.3 (0)	104.3 (0)	104.6 (0)	104.6 (0)	104.6 (0)	104.5 (0)
C-2	114.9 (1)	116.0 (1)	116.2 (1)	114.7 (1)	115.8 (1)	115.5 (1)
C-3	142.0 (0)	142.5 (0)	142.6 (0)	141.7 (0)	141.8 (0)	141.9 (0)
C-4	106.2 (1)	106.7 (1)	106.7 (1)	106.8 (1)	106.8 (1)	106.5 (1)
C-4a	156.0 (0)	154.6 (0)	154.6 (0)	154.6 (0)	154.9 (0)	154.8 (0)
C-5	99.6 (1)	99.2 (1)	99.4 (1)	99.7 (1)	98.9 (1)	99.8 (1)
C-5a	153.6 (0)	152.8 (0)	152.8 (0)	153.3 (0)	153.0 (0)	153.1 (0)
C-6	167.4 (0)	168.1 (0)	167.9 (0)	167.5 (0)	168.2 (0)	167.0 (0)
C-7	100.5 (1)	100.7 (1)	100.5 (1)	100.8 (1)	100.6 (1)	100.5 (1)
C-8	167.0 (0)	167.1 (0)	167.2 (0)	166.9 (0)	167.2 (0)	167.5 (0)
C-8a	103.9 (0)	103.6 (0)	103.5 (0)	103.3 (0)	104.0 (0)	104.0 (0)
C-9	182.0 (0)	182.0 (0)	182.1 (0)	182.0 (0)	182.6 (0)	181.9 (0)
C-11	22.5 (3)	22.5 (3)	22.5 (3)	22.2 (3)	22.5 (3)	22.6 (3)
C-12	72.6 (2)	66.1 (2)	66.1 (2)	66.0 (2)	65.9 (2)	66.0 (2)
C-13	75.9 (1)	121.6 (1)	121.8 (1)	121.5 (1)	121.8 (1)	121.6 (1)
C-14	144.4 (0)	143.2 (0)	143.0 (0)	142.8 (0)	140.0 (0)	142.0 (0)
C-15	113.0 (1)	16.6 (3)	17.6 (3)	16.6 (3)	68.6 (2)	66.1 (2)
C-16	18.9 (3)	39.8 (2)	26.0 (3)	39.9 (2)	14.9 (3)	59.9 (2)
C-17		26.5 (2)		22.4 (2)		
C-18		124.1 (1)		43.6 (2)		
C-19		132.6 (0)		70.9 (1)		
C-20		25.9 (3)		30.2 (3)		
C-21		17.6 (3)		29.6 (3)		
OCH_3	59.0 (3)			58.8 (3)		

[a] Numbers in parentheses designate the number of attached protons at the respective carbon.

The EIMS of **3** showed a $[M]^+$ peak at *m/z* 356 corresponding to the molecular formula $C_{20}H_{20}O_6$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 20 carbon signals that were sorted by DEPT experiments into Me × 2; OCH_3 × 1; OCH_2 × 1; OCH × 1; $=CH_2$ × 1; =CH × 4; =C × 9; C=O × 1 (Table 2). The 1,8-dihydroxy-6-methyl-3-oxyxanthone moiety was confirmed by the proton signals, 12.03 (1H, OH-1), 12.22 (1H, OH-8), and 2.35 (3H, s, Me-11), and by the two AB spin systems, 6.18 (1H, d, J = 2.5 Hz, H-7), 6.41 (1H, d, J = 2.5 Hz, H-5), 6.78 (1H, d, J = 1.7 Hz, H-2), 6.96 (1H, d, J = 1.7 Hz, H-4). The other proton signals were attributed to the side chain, which was identified as 2-methoxy-3-methylbut-3-enyloxy as in compound **2**. The structure was also confirmed by the analysis of the ^{13}C NMR spectrum resonances with those of similar derivatives; thus, **3** was identified as 1,8-dihydroxy-3-(2-methoxy-3-methylbut-3-enyloxy)-6-methylxanthone, a new derivative.

Compound **4** showed a $[M]^+$ peak at *m/z* 394 corresponding to the molecular formula $C_{24}H_{26}O_5$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 24 carbon signals that were sorted by DEPT experiments into Me × 4; CH_2 × 2; OCH_2 × 1; =CH × 6; =C × 10; C=O × 1 (Table 2). Compound **5** showed a $[M]^+$ peak at *m/z* 326 corresponding to the molecular formula $C_{19}H_{18}O_5$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 19 carbon signals that were sorted by DEPT experiments into Me × 3; OCH_2 × 1; =CH × 5; =C × 9; C=O × 1 (Table 2). Compound **6** showed a $[M]^+$ peak at *m/z* 426 corresponding to the molecular formula $C_{25}H_{30}O_6$, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 25 carbon signals that were sorted by DEPT experiments into Me × 4; CH_2 × 3; OCH_3 × 1; OCH_2 × 1; OC × 1; =CH × 5; =C × 9; C=O × 1 (Table 2).

Skeleton proton signals of compounds **4**−**6** were superimposable with those of the skeleton of **3** (see Experimental Section). These data were confirmed by the ^{13}C carbon resonances (Table 2), although **4**−**6** had very different side chains.

The side chain of **4** was identified as a geranyl moiety by comparison of ^{1}H NMR data with those of 3-geranyloxyemodin: δ 1.62 (3H, s, Me-21), 1.67 (3H, s, Me-22), 1.80 (3H, s, Me-15), 2.19 (4H, br s, CH_2-16 and CH_2-17), 4.60 (2H, d, J = 6.5 Hz, CH_2-12), 5.10 (1H, m, H-18), 5.58 (1H, d, J = 6.6 Hz, H-13), and confirmed by the ^{13}C NMR data. Therefore, **4** was identified as 1,8-dihydroxy-3-geranyloxy-6-methylxanthone, a new derivative.

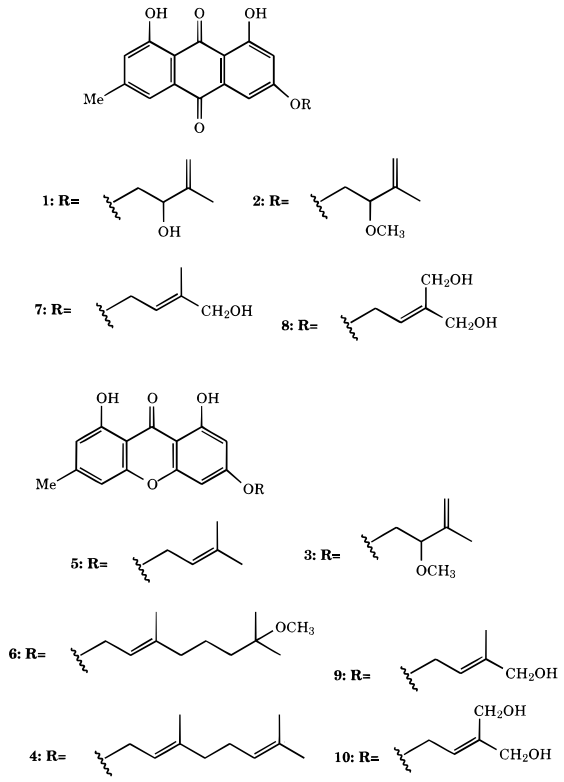
The side chain of **5** was identified as an isoprenyl moiety by comparison of ^{1}H NMR data with those of madagascin: δ 1.78 (6H, br s, Me-15 and Me-16), 2.34 (3H, s, Me-11), 4.64 (2H, d, J = 6.7 Hz, CH_2-12), 5.48 (1H, d, J = 6.7 Hz, H-13), and confirmed by the ^{13}C NMR data. Therefore, **5** was identified as 1,8-dihydroxy-3-isoprenyloxy-6-methylxanthone, a new constituent.

The side chain of **6** was identified by comparing the ^{1}H and ^{13}C NMR data with those of 3-*O*-(3,7-dimethyl-7-hydroxyoct-2-enyl)-emodin. The proton and carbon signals of the side chain of 3-*O*-(3,7-dimethyl-7-hydroxyoct-2-enyl)-emodin and **6** were the same, but the signal of the aliphatic hydroxyl at δ 5.70 in the emodin derivative was replaced by a singlet (3H) of a methoxyl at δ 3.79. These suggestions were also confirmed by the carbon resonance at 56 ppm and the other ^{13}C NMR resonances, taking into account the etherification shifts. Therefore, **6** was identified as 1,8-dihydroxy-3-(3,7-dimethyl-7-methoxyoct-2-enyloxy)-6-methylxanthone, a new derivative.

Compound **7** showed the same molecular formula as **1** ($C_{20}H_{18}O_6$, [M^+] 354 *m*/*z*) and the same anthraquinone skeleton as derivatives **1** and **2**, also deduced by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 20 carbon signals, which were sorted by DEPT experiments into Me × 2; OCH_2 × 2; =CH × 5; =C × 9; C=O × 2 (Table 1). The side chain was identified by comparing the proton shifts with those of **1** and madagascin.[4] The proton signals were identical to those of madagascin, with the exception of the vinylic methyl (3H, δ 1.65, s), which was replaced by an oxymethylenic signal (2H, δ 3.63, s). This fact was also confirmed by the presence of CH_2O (68.5 ppm, C-15) and a CH_3 (15.0 ppm, C-16) in the ^{13}C NMR spectrum.[13,14] Furthermore, the evaluation of the carbon resonances revealed a *trans* configuration of the side chain[13] and led to the identity of **7** as 3-*O*-(*E*-3-hydroxymethylbut-2-enyl)-emodin, another new derivative.

The residue obtained from the $CHCl_3$ extract was suspended in 95% EtOH to obtain an insoluble portion that was submitted to gel filtration. Purification of the eight fractions obtained yielded 11 constituents, including three new derivatives. The known compounds were identified as bianthrone A_1, β-sitosterol, 2α-hydroxyursolic acid, ursolic acid, betulinic acid, tormentic acid, 3-methylemodin, and emodin.

From fraction VIII, after repeated purifications by gel filtrations and both flash and gravity SiO gel column chromatography, the closely structurally related compounds **8**−**10** were isolated and identified. Compound **8** showed a similar molecular formula as **1** and **7** ($C_{20}H_{18}O_7$, [M^+] 370 *m*/*z*), and their ^{1}H and ^{13}C NMR data revealed the same anthraquinone skeleton, deduced also by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum revealed 20 carbon signals that were sorted by DEPT experiments into Me × 1; OCH_2 × 3; =CH × 5; =C × 9; C=O × 2 (Table 1). The side chain was identified by comparison of the proton spectrum resonances with those of **7**. All the proton signals were identical to those of **7**, with the lack of one vinylic methyl of the side chain that was replaced by an oxymethylene (δ 4.07 and 3.85). This suggestion was confirmed by the ^{13}C NMR resonances at 59.8 (C-16) and 66.1 (C-15) ppm.[13,14] Therefore, **8** was identified as 3-*O*-(3-hydroxymethyl-4-hydroxybut-2-enyl)-emodin, a new anthraquinone derivative.



Compounds **9** and **10** showed molecular formulas closely related to that of **5**, that is, $C_{19}H_{18}O_6$, [M^+] 342 *m*/*z* and $C_{19}H_{18}O_7$, [M^+] 358 *m*/*z*, respectively, due to the molecular formula of **5** plus one oxygen for **9** and two oxygens for **10**. ^{1}H and ^{13}C NMR data confirmed these suggestions and showed that **9** and **10** had the same xanthone skeleton as compound **5**, also deduced by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum of both constituents revealed 19 carbon signals that were sorted by DEPT experiments into Me × 2; OCH_2 × 2; =CH × 5; =C × 9; C=O × 1, and Me × 1; OCH_2 × 3; =CH × 5; =C × 9; C=O × 1, respectively (Table 2). Furthermore, the analysis of NMR data showed that compound **9** had the same side chain as **7**, and compound **10** had the same side chain as **8**. Therefore, **9** was identified as the new derivative 1,8-dihydroxy-3-(*E*-3-hydroxymethylbut-2-enyloxy)-6-methylxanthone, and **10** is another new compound, 1,8-dihydroxy-3-(3-hydroxymethyl-4-hydroxybut-2-enyloxy)-6-methylxanthone.

Gel filtration of the $CHCl_3$−MeOH extract gave six fractions (I−VI) from which emodin and apigenin-6-*C*-β-D-glucopyranoside were isolated and identified.

All the quinonoid constituents were submitted to preliminary *in vitro* cytogenetic assay. The investigation was performed as previously reported.[7−9,15] Results of the antimitotic activity assay are summarized in Table 3 where the mitotic index (MI) was calculated by scoring the number of mitotic figures out of the total of at least 2500 stimulated nuclei. The values of MI are expressed as the mean of two experiments. Estramustin and colcemide were used as reference compounds. Only treatment with 1,8-

Table 3. Results of the Metaphase-Blocking Activity Assay, Expressed as Mitotic Index (MI), in Human Lymphocytes Treated with Xanthone (**6**) and the Reference Compounds, Estramustin and Colcemide

compound	dose (μM)	MI[a], mean ± SD[b] (%)
negative control		20.9 ± 1.9
xanthone **6**	0.5	47.4 ± 3.8[c]
xanthone **6**	1	48.7 ± 4.2[c]
xanthone **6**	25	49.9 ± 4.2[c]
colcemide	1	70.1 ± 3.6[d]
estramustin	1	78.7 ± 3.6[d]

[a] Mitotic index is the ratio between mitotic figures and the number of stimulated nuclei. [b] Values represent the average of two experiments. [c] $p < 0.05$. [d] $p < 0.001$ the solvent control.

dihydroxy-3-(2-methoxy-3-methylbut-2-enyloxy)-6-methylxanthone (**6**) led to significantly different MI values as compared with the negative control, showing a valuable antimitotic activity (cell-cycle arrest ability). However, as expected, MI of cultures given the positive control compounds estramustine and colcemide were markedly higher compared to the untreated control.

Previously, from *V. guineensis* collected in Ivory Coast, β-sitosterol, tetra- and penta-oxygenated xanthones, vismiones, and anthranoids were isolated and identified. In our investigation on the same plant collected in Mali, 23 compounds, belonging to the classes of sterols, triterpenes, quinoids, and flavonoids, were isolated and identified. Ten of these (five xanthones and five anthraquinones) are structurally new. No vismiones and anthrones, considered by many authors[16,17] as products of chemical transformation during the isolation process, were present. All the isolated xanthones and anthraquinones showed isoprenyl chains typical of the tribe Vismiae, which includes the genera *Harungana*, *Psorospermum*, and *Vismia*.[18,19]

Furthermore, a preliminary screening on the antimitotic activity of the pure quinoids isolated evidence that the new 1,8-dihydroy-3-(2-methoxy-3-methylbut-2-enyloxy)-6-methylxanthone (**6**) had significant dose-related activity of up to 25 mg, even if the reference compounds estramustine and colcemide displayed a higher potency (see Table 3). However, this action could be interesting due to the antitumor and cytotoxic properties of some xanthones isolated from *Psorospermum febrifugum* L. roots, a species closely related to *V. guineensis*, used with analogue traditional aims and preparations[20−23] and recently employed in antileukemic therapy.[24−26]

Experimental Section

General Experimental Procedures. Analytical TLCs were carried out on Si gel 60 F_{254} precoated Al sheets and RP_{18} HPTLC plates (Merck). Compounds were visualized under UV 254 nm and by spraying with $Ce(SO_4)_2-H_2SO_4$ (Aldrich) and NTS−PEG (Aldrich) reagents. Preparative TLC was performed on Si gel 60 F_{254} precoated glass sheets. Lobar RP_8 (40−63 mm, Merck), Si gel 60 (70−230 mesh and 230−400 mesh, Merck), and Sephadex LH-20 (Pharmacia) were used for column chromatographies.

All NMR measurements were performed on a Bruker AC-200 Spectrospin spectrometer operating at 200.06 MHz for proton and 50.31 MHz for carbon spectra; the spectra were recorded in CD_3OD or $CDCl_3$; chemical shifts are given in δ values (ppm) with TMS as internal standard. DEPT, COSY, NOESY, and HETCOR spectra were recorded using standard Bruker microprograms. IR and UV spectra were recorded on Perkin-Elmer spectrophotometers, models 684 and 330, respectively; EIMS spectra were registered on a HP 5988A model (70 eV).

Plant Material. Roots (900 g) of *V. guineensis* were collected and dried in Mali on July 1995, by Prof. Keita Arouna. Dried voucher specimens are deposited in the Département de Médecine Traditionelle, Institut National de Recherche en Santé Publique, BP 1746, Bamako, Mali.

Extraction and Isolation. Powdered plant material (900 g) was extracted with *n*-hexane, $CHCl_3$, and $CHCl_3$−MeOH (9:1). The solvents were removed under reduced pressure, and the residue obtained from the $CHCl_3$ extract was suspended in EtOH 95% to obtain an insoluble portion that was further purified. All the residues (22.6, 15.0, and 18.3 g, respectively) were chromatographed on Sephadex LH-20 column, using MeOH−$CHCl_3$ mixtures as mobile phases. The fractions were collected after monitoring by TLC on Si gel using *n*-hexane−acetone and $CHCl_3$−MeOH mixtures as eluents and detected by $Ce(SO_4)_2-H_2SO_4$ spray reagent.

A part of the *n*-hexane residue (10.8 g) gave four fractions (I−IV). Fraction I (380 mg) was further purified by preparative TLC on Si gel (eluents: *n*-hexane−EtOAc, 2:1) to yield **1** (7 mg). Fraction III (4880 mg) was again submitted to a Sephadex LH-20 gel filtration (eluents: *n*-hexane−EtOAc mixtures from 1:3 to 1:40) to give seven homogeneous subfractions (IIIa−IIIg). From IIIa, by flash column chromatography (CC) on Si gel, 3-*O*-(3,7-dimethyl-6,7-dihydroxyoct-2-enyl)-emodin (10 mg) was isolated. From IIIb, by flash CC on Si gel, pure 3-geranyloxyemodin (5 mg), madagascin (30 mg), **2** (6 mg), and **3** (8 mg) were obtained. Fraction IIIc, was again purified by flash and gravity Si gel CC, to obtain pure **4** (14 mg), **5** (12 mg), and **6** (10 mg). From IIIe, by preparative TLC on Si gel, pure 3-(3,7-dimethyl-7-hydroxyoct-2-enyl)-emodin (11 mg) was obtained, while IIIf yielded **7** (13 mg).

The EtOH 95% insoluble part of the $CHCl_3$ residue (10.0 g) was submitted to Sephadex LH-20 gel filtration (eluents: CH_2Cl_2−light petroleum, 4:1) to give eight fractions (I−VIII). Fraction II (100 mg) was further purified by flash CC on Si gel (eluents: mixtures of $CHCl_3$−MeOH of increasing polarity, from 99 to 95%), to yield bianthrone A_1 (25 mg). Fraction III was further purified by preparative Si gel TLC (eluents: $CHCl_3$−MeOH, 9:1) to yield β-sitosterol (12 mg) and 2α-hydroxyursolic acid (20 mg). Fraction IV gave ursolic acid (15 mg), betulinic acid (7 mg), and tormentic acid (5 mg) after repeated purifications by gravity and flash Si gel CC (eluents: $CHCl_3$−MeOH, 9:1 and 8:2). Fraction VIII was first purified by Sephadex LH-20 CC (eluents: $CHCl_3$−MeOH, 4:1 to 1:1) to yield eight fractions (VIIIa−VIIIh). From VIIIc, by repeated purifications by gravity Si gel CC, pure **8** (14 mg) was isolated, while from VIIIe compounds **9** and **10** were isolated as a mixture (54 mg), which was separated by Sephadex LH-20 CC (10 and 18 mg, respectively). From VIIIg, 3-methylemodin (18 mg) was isolated, and emodin (22 mg) was isolated from VIIIh.

Part of the $CHCl_3$−MeOH residue (3.7 g) was submitted to Sephadex LH-20 CC (eluents: MeOH−H_2O, 4:1) to obtain six fractions (I−VI). Fraction V (315 mg), submitted to Lobar RP_8 CC (eluents: MeOH−H_2O, 7:3, 4:1, and 9:1) gave pure apigenin-6-*C*-β-D-glucopyranoside (26 mg).

Known Compounds. Quinoids and apigenin-6-*C*-β-D-glucopyranoside were identified by comparison of ^{1}H NMR, ^{13}C NMR, and UV data with those in the literature.[10,16,17,21−23] β-Sitosterol and triterpenic acids were identified by comparison of their TLC, R_f, and ^{1}H and ^{13}C NMR data with authentic samples.

3-*O*-(2-Hydroxy-3-methylbut-3-enyl)-emodin (1): isolated as a red solid; mp 193−196 °C; UV (EtOH) λ_{max} 480, 289, 260 nm; IR (Nujol) ν_{max} 3400, 1685, 1640, 1580, 1480, 1380, 1300, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.82 (3H, br s, Me-16), 2.35 (3H, s, Me-11), 4.05 (1H, dd, J = 1.7, 8.7 Hz, H-12a), 4.14 (1H, dd, J = 9.6, 8.7 Hz, H-12b), 4.46 (1H, dd, J = 1.7, 9.6 Hz, H-13), 4.97 (1H, m, H-15a), 5.11 (1H, m, H-15b), 5.60 (1H, br s, OH-13), 6.70 (1H, d, J = 2.5 Hz, H-7), 6.98 (1H, d, J = 1.7 Hz, H-2), 7.29 (1H, d, J = 2.5 Hz, H-5), 7.65 (1H, d, J = 1.7 Hz, H-4), 12.10 (1H, OH-1), 12.30 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 1; EIMS *m*/*z* 354 (10.6) with a base peak at 269; *anal.* C 67.75%, H 5.11%, 27.14%; calcd for $C_{20}H_{18}O_6$, C 67.79%, H 5.12%, O 27.09%.

3-*O*-(2-Methoxy-3-methylbut-3-enyl)-emodin (2): isolated as red-orange needles; mp 152−156 °C; UV (EtOH) λ_{max} 450, 289, 260 nm; IR (Nujol) ν_{max} 3400, 1685, 1640, 1570, 1490, 1380, 1260, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.85 (3H, br s, Me-16), 2.38 (3H, s, Me-11), 3.95 (3H, s, OMe), 4.00 (1H, dd, J = 1.8, 8.5 Hz, H-12a), 4.16 (1H, dd, J = 9.5, 8.5 Hz, H-12b), 4.40 (1H, dd, J = 1.8, 9.5 Hz, H-13), 4.90 (1H, m, H-15a), 5.00 (1H, m, H-15b), 6.70 (1H, d, J = 2.5 Hz, H-7), 6.98 (1H, d, J = 1.7 Hz, H-2), 7.29 (1H, d, J = 2.5 Hz, H-5), 7.65 (1H, d, J = 1.7 Hz, H-4), 12.10 (1H, OH-1), 12.30 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz) see Table 1; EIMS *m/z* 368 (15.0) with a base peak at 269; *anal.* C 68.43%, H 5.45%, 26.12%; calcd for $C_{21}H_{20}O_6$, C 68.47%, H 5.47%, O 26.06%.

1,8-Dihydroxy-3-(2-methoxy-3-methylbut-3-enyloxy)-6-methylxanthone (3): isolated as a yellow-orange solid; mp 195−198 °C; UV (EtOH) λ_{max} 380, 328, 258 nm; IR (Nujol) ν_{max} 3380, 1645, 1590, 1440, 1380, 1300, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.80 (3H, br s, Me-16), 2.35 (3H, s, Me-11), 3.96 (3H, s, OMe), 4.02 (1H, dd, J = 1.8, 8.5 Hz, H-12a), 4.20 (1H, dd, J = 9.6, 8.5 Hz, H-12b), 4.41 (1H, dd, J = 1.8, 9.6 Hz, H-13), 4.88 (1H, m, H-15a), 5.03 (1H, m, H-15b), 6.18 (1H, d, J = 2.5 Hz, H-7), 6.41 (1H, d, J = 2.5 Hz, H-5), 6.78 (1H, d, J = 1.7 Hz, H-2), 6.96 (1H, d, J = 1.7 Hz, H-4), 12.03 (1H, OH-1), 12.22 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 356 (18.5) with a base peak at 257; *anal.* C 67.35%, H 5.68%, 26.97%; calcd for $C_{20}H_{20}O_6$, C 67.41%, H 5.66%, O 26.94%.

1,8-Dihydroxy-3-geranyloxy-6-methylxanthone (4): isolated as yellow needles; mp 183−186 °C; UV (EtOH) λ_{max} 378, 330, 260 nm; IR (Nujol) ν_{max} 3380, 1645, 1590, 1460, 1380, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.62 (3H, s, Me-21), 1.67 (3H, s, Me-22), 1.80 (3H, s, Me-15), 2.19 (4H, br s, CH_2-16 and CH_2-17), 2.38 (3H, s, Me-11), 4.60 (2H, d, J = 6.5 Hz, CH_2-12), 5.10 (1H, m, H-18), 5.58 (1H, d, J = 6.6 Hz, H-13), 6.18 (1H, d, J = 2.5 Hz, H-7), 6.40 (1H, d, J = 2.5 Hz, H-5), 6.77 (1H, d, J = 1.8 Hz, H-2), 6.98 (1H, d, J = 1.8 Hz, H-4), 12.14 (1H, OH-8), 12.40 (1H, OH-1); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 394 (12.9) with a base peak at 257; *anal.* C 73.00%, H 6.66%, 20.34%; calcd for $C_{24}H_{26}O_5$, C 73.08%, H 6.64%, O 20.28%.

1,8-Dihydroxy-3-isoprenyloxy-6-methylxanthone (5): isolated as yellow solid; mp 201−204 °C; UV (EtOH) λ_{max} 380, 328, 260 nm; IR (Nujol) ν_{max} 3380, 1645, 1590, 1480, 1350, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.78 (6H, br s, Me-15, Me-16), 2.34 (3H, s, Me-11), 4.64 (2H, d, J = 6.7 Hz, CH_2-12), 5.48 (1H, d, J = 6.7 Hz, H-13), 6.20 (1H, d, J = 2.5 Hz, H-7), 6.38 (1H, d, J = 2.5 Hz, H-5), 6.72 (1H, d, J = 1.7 Hz, H-2), 6.94 (1H, d, J = 1.7 Hz, H-4), 12.08 (1H, OH-1), 12.23 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 326 (20.6) with a base peak at 257; *anal.* C 69.90%, H 5.57%, 24.53%; calcd for $C_{19}H_{18}O_5$, C 69.93%, H 5.56%, O 24.51%.

1,8-Dihydroxy-3-(3,7-dimethyl-7-methoxyoct-2-enyloxy)-6-methylxanthone (6): isolated as a yellow solid; mp 224−228 °C; UV (EtOH) λ_{max} 382, 330, 260 nm; IR (Nujol) ν_{max} 3420, 1645, 1580, 1480, 1350, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.25 (6H, br s, Me-20, Me-21), 1.60 (4H, m, CH_2-17, CH_2-18), 1.80 (3H, s, Me-15), 2.12 (2H, t, CH_2-16), 2.39 (3H, s, Me-11), 3.79 (3H, s, OMe), 4.70 (2H, d, J = 7.0 Hz, CH_2-12), 5.49 (1H, d, J = 7.0 Hz, H-13), 6.18 (1H, d, J = 2.5 Hz, H-7), 6.40 (1H, d, J = 2.5 Hz, H-5), 6.80 (1H, d, J = 1.7 Hz, H-2), 6.96 (1H, d, J = 1.7 Hz, H-4), 12.05 (1H, OH-1), 12.20 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 426 (9.9) with a base peak at 257; *anal.* C 70.34%, H 7.10%, 22.56%; calcd for $C_{25}H_{30}O_6$, C 70.40%, H 7.09%, O 22.51%.

3-*O*-(*E*-3-Hydroxymethylbut-2-enyl)-emodin (7): isolated as an orange solid; mp 195−199 °C; UV (EtOH) λ_{max} 438, 285, 262, 258 nm; IR (Nujol) ν_{max} 3420, 1680, 1630, 1610, 1600, 1330, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.82 (3H, br s, Me-16), 2.40 (3H, s, Me-11), 3.63 (2H, s, CH_2-15), 4.60 (2H, br d, J = 6.9 Hz, CH_2-12), 5.20 (1H, br t, J = 6.9 Hz, H-13), 6.62 (1H, d, J = 2.5 Hz, H-7), 6.99 (1H, d, J = 1.7 Hz, H-2), 7.34 (1H, d, J = 2.5 Hz, H-5), 7.63 (1H, d, J = 1.7 Hz, H-4), 12.00 (1H, OH-1), 12.20 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 1; EIMS *m/z* 354 (25.3) with a base peak at 269; *anal.* C 67.72%, H 5.13%, 27.15%; calcd for $C_{20}H_{18}O_6$, C 67.79%, H 5.12%, O 27.09%.

3-*O*-(3-Hydroxymethyl-4-hydroxybut-2-enyl)-emodin (8): isolated as red-orange needles; mp 223−226 °C; UV (EtOH) λ_{max} 452, 286, 262 nm; IR (Nujol) ν_{max} 3420, 1680, 1630, 1610, 1600, 1330, 1260, 1150, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 2.38 (3H, s, Me-11), 3.85 (2H, s, CH_2-15), 4.02 (2H, s, CH_2-16), 4.64 (2H, br d, J = 6.8 Hz, CH_2-12), 5.24 (1H, br t, J = 6.8 Hz, H-13), 6.60 (1H, d, J = 2.5 Hz, H-7), 7.00 (1H, d, J = 1.8 Hz, H-2), 7.36 (1H, d, J = 2.5 Hz, H-5), 7.66 (1H, d, J = 1.8 Hz, H-4), 12.04 (1H, OH-1), 12.24 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 1; EIMS *m/z* 370 (9.9) with a base peak at 269; *anal.* C 64.79%, H 4.91%, 30.30%; calcd for $C_{20}H_{18}O_7$, C 64.86%, H 4.90%, O 30.24%.

1,8-Dihydroxy-3-(*E*-3-hydroxymethylbut-2-enyloxy)-6-methylxanthone (9): isolated as a yellow solid; mp 234−239 °C; UV (EtOH) λ_{max} 380, 320, 258 nm; IR (Nujol) ν_{max} 3380, 1645, 1590, 1480, 1350, 1200, 1150, 1080 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.86 (3H, br s, Me-16), 2.38 (3H, s, Me-11), 3.65 (2H, s, CH_2-15), 4.58 (2H, br d, J = 7.0 Hz, CH_2-12), 5.22 (1H, br t, J = 7.0 Hz, H-13), 6.12 (1H, d, J = 2.5 Hz, H-7), 6.34 (1H, d, J = 2.5 Hz, H-5), 6.79 (1H, d, J = 1.7 Hz, H-2), 6.93 (1H, d, J = 1.7 Hz, H-4), 12.05 (1H, OH-1), 12.15 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 342 (23.8) with a base peak at 257; *anal.* C 66.63%, H 5.30%, 28.07%; calcd for $C_{19}H_{18}O_6$, C 66.66%, H 5.30%, O 28.04%.

1,8-Dihydroxy-3-(3-hydroxymethyl-4-hydroxybut-2-enyloxy)-6-methylxanthone (10): isolated as a yellow solid; mp 246−252 °C; UV (EtOH) λ_{max} 380, 320, 258; IR (Nujol) ν_{max} 3380, 1645, 1590, 1480, 1350, 1200, 1150, 1100, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.85 (3H, br s, Me-16), 2.38 (3H, s, Me-11), 4.00 (1H, dd, J = 1.9, 8.8 Hz, H-12a), 4.10 (1H, dd, J = 9.6, 8.8 Hz, H-12b), 4.44 (1H, dd, J = 1.9, 9.6 Hz, H-13), 5.00 (1H, m, H-15a), 5.09 (1H, m, H-15b), 5.60 (1H, br s, OH-13), 6.15 (1H, d, J = 2.5 Hz, H-7), 6.39 (1H, d, J = 2.5 Hz, H-5), 6.77 (1H, d, J = 1.8 Hz, H-2), 6.92 (1H, d, J = 1.8 Hz, H-4), 12.12 (1H, OH-1), 12.35 (1H, OH-8); ^{13}C NMR ($CDCl_3$, 200 MHz), see Table 2; EIMS *m/z* 358 (26.9) with a base peak at 257; *anal.* C 63.60%, H 5.07%, 31.33%; calcd for $C_{19}H_{18}O_7$, C 63.68%, H 5.06%, O 31.25%.

Cell Cultures. Heparinized blood samples from one healthy nonsmoking human male donor were used during all experiments. Each culture consisted of 0.3 mL whole blood and 4.7 mL of Ham's F10 medium (ICN, Irvine, CA) supplemented with 10% fetal bovine serum (also from ICN) and containing 1.5% phytohemagglutinin (PHA, Wellcome), antibiotics (100 IU/mL penicillin (Gibco, B. R. L.), and 100 mg/mL streptomycin (Gibco, B. R. L.). Cultures were kept at 37 °C during the whole incubation time (72 h). Cytochalasin B (6 mg/mL, Sigma) was added at 44 h. Before treatment, each compound was dissolved in DMSO 0.37%, and the test was performed at 0.05, 0.5, 1.0, 5.0, 50, 100, and 250 mg/mL.

Slide Preparation and Slide Scoring. Lymphocyte harvesting was performed as follows: cultures were centrifuged at 2300 rpm for 4 min; the supernatant was then discarded, and the cells were resuspended in 10 mL of KCl 0.75 M to lysate the erythrocytes. After a few minutes, cells were treated with 4 mL of a solution of MeOH−HOAc (3:5, v/v, fixative). After a second centrifugation at 2300 rpm for 4 min, the supernatant was then discarded, and the cells were resuspended in 5 mL of pure MeOH. Then cells were washed twice with MeOH−HOAc (5:1, v/v). After a final centrifugation, cells were resuspended in 0.5 mL of fixative and thoroughly mixed using the tip of a Pasteur pipet and dropped from a few centimeters onto wet clean slides. Of each culture two or three slides were prepared. Air-dried slides were stained with 3% Giemsa (Merck, Darmstadt, Germany) in distilled water for 10 min and then mounted in Eukitt.

Slides were coded and scored under 400× magnification. Criteria for identifying micronuclei were those described by Fenech.[7] Moreover, out of a total of 1000 cells, the percentages of mono-, bi-, tri- and tetra-nucleated (multinucleated) cells were scored.

Statistical Analyses. Regression lines on dose vs percentage of binucleated cells were calculated for each compound. For all compounds the induction of micronucleated lymphocytes was compared statistically to that of the control by means of Fisher's exact test.

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