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TETRAHEDRON

Five New Steroids from *Solanum nudum*

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Abstract : Five new steroids, the cholest-4-ene-3,22-diones : tumacone A (**1**), tumacone B (**2**), tumacoside A (**3**), tumacoside B (**4**), and a furostenone : tumaquenone (**5**), besides diosgenone (**6**), were isolated from the aerial parts of *Solanum nudum*. Their structures were determined by 2D NMR, MS analyses and chemical correlations. Steroid **3** and **5** displayed *in vitro* antimalarial activity against a *Plasmodium falciparum* chloroquine-resistant FCB-1 strain (IC_{50} 27 and 16 μM). The observed stereodependent cyclization into spiroketals of two 16-O isomers is discussed. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords : Solanum nudum ; cholest-4-en-3,22-dione ; steroids ; tumacones A and B ; tumacosides A and B ; tumaquenone ; antimalarial ; stereodependent cyclization.

Solanum nudum (Solanaceae) is a plant growing in South America and used in the Colombian Pacific area for treatment of fevers associated with malaria. The fruits of this plant were previously shown to contain a steroidal alkaloid, solanudine.[1] *In vitro* screening of the ethanol extract of *S. nudum* showed promising antimalarial potency. By bioassay-guided fractionation of MeOH extracts of the aerial parts, we isolated five new steroids and elucidated the structure of four cholest-4-ene-3,22-diones: tumacone A (**1**), tumacone B (**2**), tumacoside A (**3**) and tumacoside B (**4**), as well as a furostenone, tumaquenone (**5**) and known diosgenone (**6**).[2]

Interestingly enough, we noticed, in *S. nudum*, the presence of two 16-C located stereoisomeric groups: 16α-oxygenated 22-ketosteroids **1**, **2**, **3** and 4 on one hand, 16β-oxygenated furostane **5** (hemiketal) and spirostane **6** (spiroketal), on another. These products allowed rationalization of a stereodependent cyclization of the 16β-oxygenated 22-ketosteroids.

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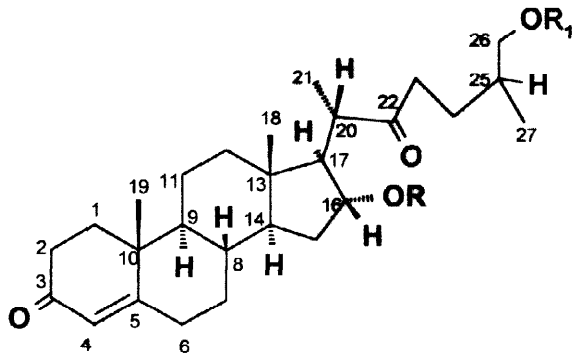
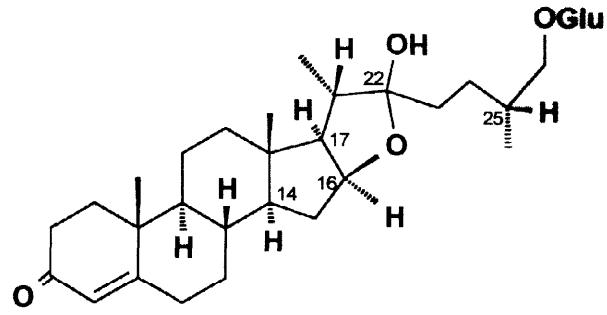




Tumaquenone **5**

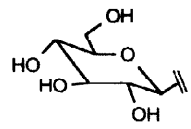
Tumacone A **1**: R=Ac, R_1=H

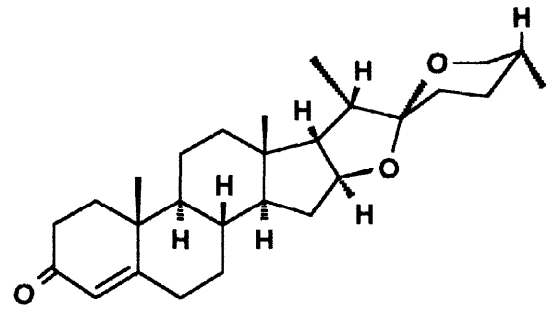
Diacetyltumacone **1a**=**2a**: R=R_1=Ac

Tumacone B **2** : R=R_1=H

Tumacoside A **3** : R=Ac, R_1=

Tumacoside B **4** : R=H, R_1=





Diosgenone **6**

Tumacone A (**1**), $[\alpha]_D$ -34.2°, had the molecular formula $C_{29}H_{44}O_5$ as deduced from its HRFABMS: *m/z* 495.3109 $[M+Na]^+$, supported by the ^{13}C NMR spectrum (Table 1). The IR spectrum of **1** showed absorption bands characteristic of ketone (1736, 1677 cm^{-1}) and hydroxyl (3434 cm^{-1}) groups. The ^{13}C NMR spectra of **1** displayed 29 carbon signals mainly in the sp^3 C field. The DEPT and ^1H-^{13}C HETCOR spectra revealed the presence of five methyls (one being acetoxyl), nine methylenes, one oxymethylene, eight methines (one olefinic) and six quaternary carbons (four sp^2 C). The ^1H NMR (Table. 2) of **1** corroborated the presence of five methyl groups : three singlets at δ 0.73, 1.15 and 1.92 and two doublets at δ 0.82 and 1.05. Hence, the presence of a cholestane skeleton was suggested and confirmed by ^1H and ^{13}C 2D NMR analyses. HMBC long-range correlations (Fig. 1) of C-3 and C-4 clearly indicated the existence of an α,β-unsaturated carbonyl system (δ_{CO} 199.5, δ_C 170.8, δ_C 123.9 and δ_H 5.69) on a choles-4-en-3-one skeleton as well as an acetyl group (δ_{CO}171.0, δ_C 21.2 and δ_H 1.92) attached to 16-O, giving a cross peak with H-16. HMBC experiments allowed also to assign the resonance at δ 213.7 to a 22-ketone group by connection with H-20 and CH_3-21. The 26-hydroxyl was deduced from the observation of a 3-(hydroxymethyl)butyl spin system of C-23 to C-27, in the COSY spectrum. The EIMS of steroid **1** (Fig. 2) showed two diagnostic fragments at *m/z* 115 and 358 produced by cleavage between C-20 and C-22, in agreement with the 22-carbonyl assignment. 16-O-Acetyl steroidal fragments at *m/z* 358 and 329 gave further two peaks at *m/z* 298 and 269 by loss of acetic acid. On the NOESY spectrum of acetylated **1a** (Fig. 3), cross-peaks between H-20 and both H-16 and CH_3-18 as well as between CH_3-18 and CH_3-19, H-8 and H-16, indicated their β-orientation. Hence, compound **1**, named tumacone A, was 16α-acetoxy-26-hydroxycholest-4-ene-3,22-dione.

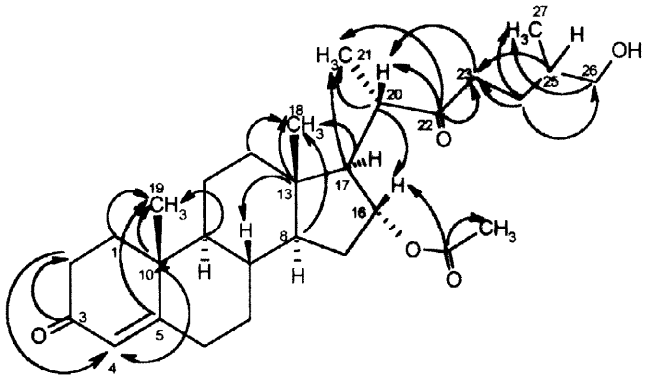


Fig. 1. HMBC correlations of tumacone A **1**.

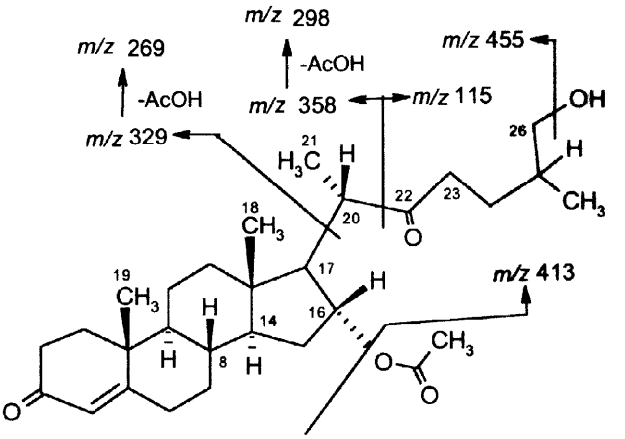


Fig. 2. EIMS fragmentations of **1**.

Tumacone B (**2**), $[\alpha]_D$ +10.6°, was isolated as a pale yellow viscous mass which analyzed for $C_{27}H_{42}O_4$ by HRFABMS (*m/z* 453.2950 $[M+Na]^+$) and ^{13}C NMR spectrum. The ^1H and ^{13}C NMR spectra of **2** exhibited signals quite similar to those of **1**, but lacking the acetyl group. The signal of C-16 at δ 78.5 of **1** was shifted to δ76.0 and H-16 at δ4.85 to δ3.65 ; these differences arose from deacetylation of compound **1** into 16-hydroxy steroid.

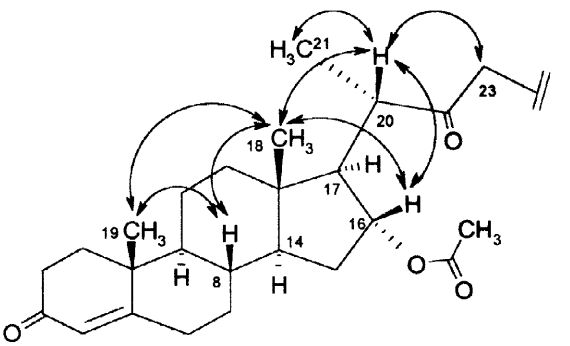


Fig. 3. Selected NOEs of **1a**.

Acetylation of **2** gave the diacetyl derivative **2a**, $[\alpha]_D$ -34.7°, identical to the acetyl derivative **1a**. Compound **2** thus possessed the same configuration as **1**. Hence, the structure assigned to tumacone B (**2**) was 16α,26-dihydroxycholest-4-ene-3,22-dione.

Tumacoside A (**3**), $[\alpha]_D$ -25.3°, possessed the molecular formula $C_{35}H_{54}O_{10}$ obtained from HRFABMS: *m/z* 657.3652, $[MNa]^+$. The ^1H and ^{13}C NMR spectra displayed the resonances of a sugar in the oxymethine field, in addition to those of tumacone A (**1**). The chemical shift of the anomeric carbon at δ 103.1 and the coupling constant (8 Hz) of the anomeric proton (δ4.19) suggested the hexose was glucose. The HMBC correlation between H-1' and C-26 allowed to attach the sugar to 26-O. The H-16 in the triplet at δ 4.83 was geminal to an acetoxyl group, confirmed by HMBC connection. In the NOESY spectrum, H-16 showed cross-peaks with H-20 and methyl-18, configurated β. Action of β-D-glucosidase on tumacoside A (**3**) furnished D-glucose and tumacone A (**1**). From these results, the structure of tumacoside A (**3**) was established as 16α-acetoxy-26-*O*-β-*D*-glucopyranosyloxycholest-4-ene-3,22-dione.

The molecular formula of tumacoside B (**4**) was $C_{33}H_{52}O_9$ deduced from HRFABMS, *m/z* 615.3467, $[MNa]^+$, one acetyl group C_2H_2O less than tumacoside A (**3**). The H-16 was shielded to δ3.83 from δ4.83 of compound **3**, indicating deacetylation at 16-OH. Two protons at 26 were distinguished by the presence of a glucosyl group and the C-26 was correlated with the anomeric proton in the HMBC spectrum. Tumacoside B (**4**) furnished D-glucose and tumacone B (**2**) by action of β-D-glucosidase. Hence, tumacoside B (**4**) was 26-*O*-β-*D*-glucopyranosyloxy-16α-acetoxycholest-4-ene-3,22-dione.

Table 1. ^{13}C-NMR Data for Compounds **1** - **6** ($CDCl_3$, 75 MHz).

C n°	1	2	1a=2a	3	4	5	6
1	35.5	35.2	35.5	35.4	35.5	35.4	35.5
2	33.8	33.5	33.8	34.0	33.7	33.5	33.8
3	199.5	199.4	199.3	200.1	200.0	200.6	199.3
4	123.9	123.4	123.9	123.7	123.6	123.3	123.7
5	170.8	171.2	170.6	171.3	171.9	172.6	171.0
6	32.6	32.4	32.5	32.7	32.7	32.6	32.6
7	31.7	31.5	31.6	31.6	31.7	31.8	32.0
8	34.9	34.5	34.8	34.8	34.8	34.9	35.0
9	53.4	53.1	53.4	53.3	53.3	53.5	53.6
10	38.5	38.2	38.4	38.5	38.4	38.5	38.5
11	20.6	20.3	20.5	20.5	20.5	20.5	20.7
12	39.2	39.2	39.2	39.2	39.4	39.2	39.5
13	43.5	43.6	43.4	43.6	43.8	40.4	40.2
14	52.7	52.1	52.7	52.6	52.4	55.2	55.5
15	34.1	35.1	33.9	33.7	35.3	31.3	31.6
16	78.5	76.0	78.5	78.6	75.7	80.7	80.4
17	57.7	62.1	57.7	57.7	62.2	62.2	61.9
18-Me	13.2	13.3	13.1	13.1	13.4	16.0	16.2
19-Me	17.3	17.0	17.2	17.2	17.1	17.0	17.2
20	47.6	48.3	47.8	47.6	48.8	39.7	41.5
21-Me	16.4	16.5	16.2	17.2	16.4	15.0	14.4
22	213.7	216.9	212.7	214.3	217.4	110.4	109.1
23	39.2	38.4	38.9	39.2	38.1	35.2	31.2
24	25.9	26.6	26.6	26.6	27.0	26.8	28.6
25	35.1	34.7	32.0	32.5	32.7	32.3	30.1
26	67.2	66.9	68.8	74.8	75.1	75.0	66.7
27-Me	16.5	16.3	16.6	16.3	16.5	16.5	17.0
16-OAc	171.0	-	170.5	171.5	-	-	-
	21.2	-	21.1	21.1	-	-	-
26-OAc	-	-	171.1	-	-	-	-
	-	-	20.8	-	-	-	-
26-OGlu							
1'	-	-	-	103.1	103.1	103.0	-
2'	-	-	-	73.6	73.5	73.4	-
3'	-	-	-	76.2	76.0	75.6	-
4'	-	-	-	69.9	69.6	69.8	-
5'	-	-	-	76.2	76.2	76.1	-
6'	-	-	-	61.7	61.2	61.4	-

Table 2. ^{1}H-NMR Data for Compounds **1** - **6** ($CDCl_3$, 300 MHz).

C n°	**1**	**2**	**1a=2a**	**3**	**4**	**5**	**6**
1	1.65	1.50	1.64	1.62	1.64	1.58	1.60
	1.95	1.88	1.76	1.93	1.76	1.58	1.95
2	2.32	2.22	2.32	2.28	2.27	2.23	2.28
4	5.69 s	5.52 s	5.65 s	5.60 s	5.65 s	5.60 s	5.65 s
6	2.23	2.13	2.21	2.19	2.17	2.16	2.19
	2.36	2.30	2.36	2.25	2.23	2.32	2.35
7	1.02	0.96	0.99	1.00	1.01	0.92	0.95
	1.70	1.69	1.70	1.70	1.76	1.73	1.78
8	1.50	1.42	1.48	1.48	1.48	1.62	1.65
9	0.95	0.88	0.94	0.93	0.93	0.80	0.85
11	1.35	1.24	1.33	1.29	1.31	1.41	1.33
	1.55	1.43	1.53	1.42	1.65	1.41	1.47
12	1.34	1.27	1.37	1.35	1.30	1.04	1.12
	1.97	1.78	1.93	1.89	1.89	1.66	1.67
14	1.32	1.33	1.30	1.27	1.36	1.01	1.05
15	1.45	1.45	1.45	1.40	1.32	1.18	1.25
	1.69	1.80	1.72	1.72	1.85	1.86	1.95
16	4.85 t, 8	3.65dd,7;14	4.83 t, 8	4.83 t, 8	3.83	4.28dd,7;14	4.32dd,7;14
17	1.84	1.48	1.59	1.80	1.48	1.67	1.70
18-Me	0.73 s	0.62 s	0.74 s	0.70 s	0.68 s	0.70 s	0.76 s
19-Me	1.15 s	1.05 s	1.12 s	1.10 s	1.10 s	1.06 s	1.12 s
20	2.65	2.58	2.60	2.62	2.62	1.92	1.80
21-Me	1.05 d, 7	1.02 d, 7	1.05 d, 7	1.02 d, 7	1.08 d, 7	0.87 d, 7	0.88 d, 7
23	2.45 t, 7.8	2.39	2.40	2.40	2.48 t, 7.8	1.62	1.53
	2.45 t, 7.8	2.53	2.45	2.45	2.48 t, 7.8	1.90	1.55
24	1.32	1.32	1.25	1.23	1.36	1.20	1.35
	1.51	1.55	1.57	1.59	1.66	1.53	1.55
25	1.53	1.46	1.68	1.65	1.60	1.64	1.56
26	3.40 d, 7	3.20 d, 7	3.80	3.28	3.30	3.22	3.28
	3.40 d, 7	3.20 d, 7	3.83	3.60	3.68	3.64	3.39
27-Me	0.82 d, 7	0.77 d, 7	0.86 d, 7	0.82 d, 7	0.80 d, 7	0.77 d, 7	0.72 d, 7
16-OAc	1.92 S	-	1.89 s	-	1.90 s	-	-
26-OAc	-	-	1.98 s	-	-	-	-
26-OGlu							
1'				4.19 d, 8	4.20 d, 8	4.12 d, 8	
2'				3.20	3.20	3.18	
3'				3.38	3.34	3.32	
4'				3.39	3.35	3.32	
5'				3.20	3.20	3.19	
6'				3.68	3.69	3.66	
				3.77	3.80	3.70	

Tumaquenone **5**, analyzed for $C_{33}H_{52}O_9$ by HRFABMS, was an isomer of steroid **4**. The ^{13}C NMR spectra showed a dioxygenated C (δ 110.4) lacking 22-C=O resonance. The NOESY spectrum presented cross-peaks between H-16 and H-17α, indicating a 16β-O-configuration. β-D-glucosidase produced cyclization of tumaquenone 5 into diosgenone **6**, a spiroketal. Thus compound **5** was 26-O-β-D-glucopyranosyloxy-25(*R*)-furost-4-en-3-one.

The NOESY spectrum of diosgenone **6** , 25(*R*)-spirost-4-en-3-one, displayed correlations between H-16 and H-17α and H-14α on the α side and between CH_3-18 and CH_3-19, H-8 and H-20 (CH_3-21) on the β side. The chemical shifts of CH-25 (δ_H 1.56, δ_C 30.1) and CH_3-27 (δ_H 0.72, δ_C 17.0) indicated 25*R* configuration (25S: δ_{C25} 26 and δ_{C27}16).[3]

These steroids may be classified into two stereochemically distinct groups: 16Hα and 16Hβ. *S. nudum* thus produced mainly 16β- as well as small amount of 16α-oxygenated steroids. Only 16β-OH with simultaneous presence of 22-C=O, never found in nature until now, induced spontaneous cyclization into hemiketal, the corresponding furostane and further, into spiroketal in presence of 26-OH.[4] The furostenone, tumaquenone **5**, was thus transformed into a spiroketal, diosgenone **6**, a major product in *S. nudum*

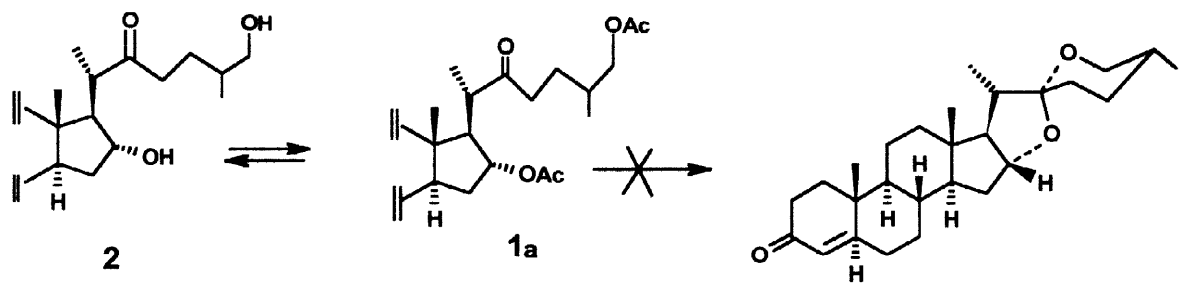


Fig. 4. Reaction of 16α-acetoxycholest-4-ene-3,22-diones.

16β-O-, 26-O- diacetylcholestan-22-one, produced by opening of the spirostane by action of BF_3-Ac_2O, was described to cyclize into starting spiroketal by saponification with KOH/MeOH,[5] whereas 16α-O-,26-O-diacetate **1a** returned to the starting tumacone **2**: 16α,26-diol, on saponification (Fig. 4). Both 22-ketone and free non-cyclizable 16α-OH groups are thus found in the steroids of *S. nudum* and other natural sources : *Solanum abutiloides* [6], and *Fevillea cordifolia* [7].

In order to understand the stereo-dependent cyclization into spiroketal, the conformation of acetyltumacone A (**1a**), diosgenone (**6**) and the 16β-O-isomer of **1a**, presenting closest 16-O / 22-C were obtained by molecular dynamic simulation and energy minimization calculations (Hyperchem program package)[8]. The dihedral angle O16-C16-C17-C20 on the computed structure was 36.9° for equatorial 16β-O steroid; 15.6° for the spirostane **6**, while the dihedral angle for 16α-O steroid **1a** was –84.9°, too strained to allow 16α-O / C-22 linkage to be established.

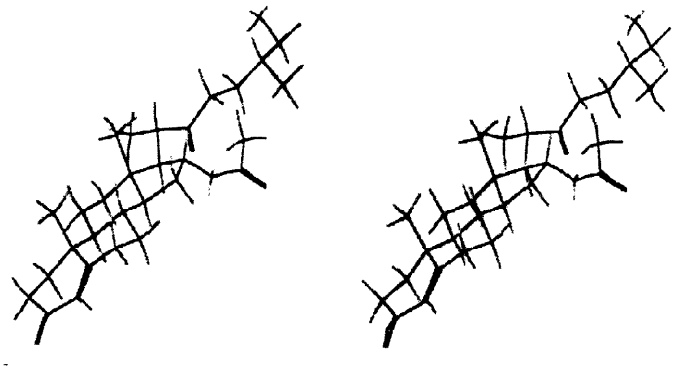


Fig. 5. Stereoview of the most stable energy minimized structure of tumacone A (**1**).

Noteworthy was the chemical shift of the 22- ketone influenced by the 16α-O acetyl group: the signal near δ 217 in 16α-hydroxy compounds **2**, **4** and 16β-glycosyl steroids [4] was shielded to about δ214 in 16α-acetoxyl steroids **1** and **3**. The MM2 energy minimized structure of **1** (Fig. 5) showed that C-22 was found in a positive anisotropic area of the 16α-acetoxy carbonyl group.

In addition to the biogenetical and stereochemical interest of *S. nudum* steroids, new natural sources and synthesis[9] of cholest-4-en-3-one are far-reaching, since this structural feature constitute the A-ring of major steroidal hormones, such as testosterone, progesterone and aldosterone, as well as of corticosteroids such as cortisone and algestone.

Tumacoside A (**3**) and tumaquenone (**5**) displayed antimalarial activity *in vitro* against *Plasmodium falciparum,* a chloroquine-resistant FCB-1 strain, with IC_{50} 27 and 16 μM (17.02 and 9.54 μg/mL), respectively. Tumacone A (**1**) and B (**2**) were inactive (IC_{50} 614 and >1000 μg/mL).

EXPERIMENTAL

General Methods. NMR spectra were measured at 300 MHz for ^1H and 75 MHz for ^{13}C on a Bruker AC300 spectrometer. HRFABMS were obtained on a ZAB-HF mass spectrometer; optical rotations on a Perkin-Elmer 141 polarimeter; FT-IR spectra on a Nicolet Impact 400D spectrometer.

Extraction and isolation. *S. nudum* was collected in Tumaco (Colombia) and a voucher specimen deposited in the Herbarium of the University of Antioquia (HUA 61181). The grounded air-dried aerial parts (1.2 kg) were extracted exhaustively by percolation at room temp. with petroleum ether, then with MeOH. The MeOH extracts, concentrated in vacuo, were partitioned between H_2O and AcOEt, to furnish an AcOEt extract (90 g). 30 g of AcOEt extract was subjected to cc over Si gel eluting with a gradient of CH_2Cl_2/AcOEt to yield seven fractions. The residue of fr. 2, active against *P. falciparum* FCB-1 strain (30 % of growth inhibition at 100 μg/mL), was further fractionated repeatedly on Si gel cc eluting with a CH_2Cl_2/ MeOH gradient, to afford compounds: **1** (214 mg), **2** (240 mg), **3** (20 mg), **4** (20 mg), **5** (15 mg) and **6** (1.16 g).

Acetylation. Compound **1** or **2** (5 mg) was kept overnight at 20 °C with Ac_2O (1 mL) and pyridine (1 mL). The excess Ac_2O was decomposed by addition of H_2O and the acetylated compound was extracted with CH_2Cl_2 and purified on TLC.

Enzymatic hydrolysis. Steroid **3**, **4** or **5** (2 mg) was incubated at 37 °C, 24h, with β-D-glucosidase (1 mg) in acetic buffer, pH 5, 10 mM (1 mL). The reaction mixture was extracted by CH_2Cl_2 to obtain compound **1**, **2** or **6** respectively (TLC, ^1H NMR). The aq. phase was analyzed by co-TLC with D-glucose on Si gel (CH_2Cl_2 : MeOH= 75/25).

Tumacone A (1). $[\alpha]_D$ -34.2° (MeOH, c 1.0). IR (film) ν_{max} cm^{-1}: 3434, 2927, 1736, 1677, 1650, 1250; 1032. HRFABMS: *m/z* 495.3109 $[M+Na]^+$, calcd 495.3086 for $C_{29}H_{44}O_5Na$. EIMS: *m/z* 472 (M^+), 455, 413, 358, 329, 298, 269, 115, 97.

Tumacone B (2). $[\alpha]_D$ +10.6° (MeOH, c 1.0). IR (film) ν_{max} cm^{-1}: 3440, 2933, 1664, 1617. HRFABMS: *m/z* 453.2950 $[M+Na]^+$, calcd 453.2981 for $C_{27}H_{42}O_4Na$. EIMS: *m/z* 430 (M^+), 413, 397, 343, 316, 287, 115, 97.

Acetyltumacone A (1a = 2a). $[\alpha]_D$ -34.7° (MeOH, c 1.0). IR (film) ν_{max} cm^{-1}: 2940, 1740, 1677, 1618, 1242, 1038.

Tumacoside A (3). $[\alpha]_D$ -25.3° (MeOH, c 1.0). IR (film) ν_{max} cm^{-1}: 3434, 2927, 1740, 1677, 1623, 1249, 1032. HRFABMS: *m/z* 657.3652 $[M+Na]^+$, calcd 657.3615 for $C_{35}H_{54}O_{10}Na$.

Tumacoside B (4). $[\alpha]_D$ -9° (MeOH, c 1.0). HRFABMS: *m/z* 615.3467 $[M+Na]^+$, calcd 615.3509 for $C_{33}H_{52}O_9Na$.

Tumaquenone (5). $[\alpha]_D$ +2.7° (MeOH, c 1.0). IR (film) ν_{max} cm^{-1}: 3420, 2927, 1682 1213. HRFABMS: *m/z* 615.3528 $[M+Na]^+$, calcd 615.3509 for $C_{33}H_{52}O_9Na$.

Diosgenone (6). $[\alpha]_D$ -8.8° ($CHCl_3$, c 1.0).

***In vitro* antimalarial activity.**[10] *P. falciparum*, the chloroquine-resistant FCB-1 strain, was maintained on human type O^+ erythrocytes in RPMI 1640 culture medium system.[11] The diluted erythrocyte suspensions (0.8 % parasitemia and 5 % hematocrit) were incubated 24 h, 37° C in 5 % O_2, 5 % CO_2 and 90 % N_2 with test compound, co-precipitated with polyvinylpyrrolidone (1/4), in 96 well microculture plate. Growth inhibition was determined by the method previously described.[12]

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