Notes

Bioactive Acridone Alkaloids from *Swinglea glutinosa*

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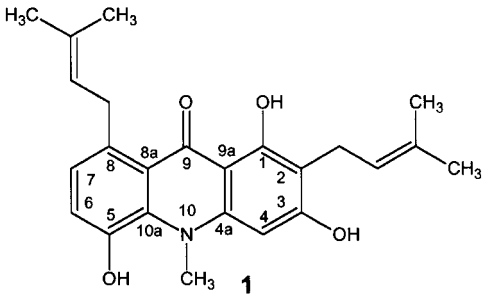
A new prenylated acridone alkaloid, 1,3,5-trihydroxy-2,8-bis(3-methylbut-2-enyl)-10-methyl-9-acridone (**1**), was isolated from the stembark of *Swinglea glutinosa*, along with three known acridone alkaloids, 5-hydroxynoracronycine (**2**), 1,3,5-trihydroxy-4-methoxy-2-(3-methylbut-2-enyl)-10-methyl-9-acridone (**3**), and 1,3,5-trihydroxy-4-methoxy-10-methylacridone (**4**). The isolated alkaloids were assessed in vitro against chloroquine-sensitive and -resistant *Plasmodium falciparum* strains and for cytotoxicity using HeLa cells.

Swinglea glutinosa Merr. (Rutaceae) is a small tree native to South East Asia, introduced and cultivated in Colombia to make living fences. A previous report on this plant mentions the presence of an alkaloid and triterpenes in the fruits.[1] Extracts of the stem bark of this plant have been previously assessed for antiplasmodial activity, showing promising activity in vitro against *Plasmodium falciparum*.[2] In continuation of our investigations on bioactive alkaloids from Central and South American plants,[3,4] we report in this publication the isolation and structure elucidation of one new and three known acridone alkaloids from the stembark of this species and their assessment for antiplasmodial activity and cytotoxic effects.

The methanol extract of the stem bark of *S. glutinosa* was subjected to several combinations of silica gel and Sephadex LH20 liquid chromatography affording four acridone alkaloids (**1**−**4**). Compounds **2**, **3**, and **4** were characterized as 5-hydroxynoracronycine, 1,3,5-trihydroxy-4-methoxy-2-(3-methylbut-2-enyl)-10-methyl-9-acridone and 1,3,5 trihydroxy-4-methoxy-10-methylacridone (also called citrusinine II), respectively, by analysis of their NMR spectra and comparison with reference data.[5,6]

^{1}H NMR of **1** indicated a chelated hydroxy proton (δ 15.07), two AB type aromatic protons [δ 7.07 (1H, d, J = 8.2 Hz, H-6), 6.84 (1H, d, J = 8.2 Hz, H-7)], an aromatic proton [δ 6.28 (1H, s, H-4)], and an *N*-methyl group (δ 3.77). The ^{1}H NMR signals of two trisubstituted double bonds [δ 5.33 (1H, br tr, J = 6.87 Hz, H-17), 5.21 (1H, br tr, J = 6.83 Hz, H-12)], two methylene protons [δ 3.93 (2H, d, J = 6.96 Hz, H-16), 3.21 (2H, d, J = 6.57 Hz, H-11)], and four vinyl methyl groups [δ 1.73 (3H, s, H-15 *cis*), 1.68 (3H, s, H-20 *cis*), 1.64 (3H, s, H-19 *trans*), 1.61 (3H, s, H-14 *trans*)] suggested two prenyl groups (3-methylbut-2-enyl) at positions 2 and 8. In HMBC, H-11 correlated with C-1/C-2/C-3, 1-OH with C-9a/C-1/C-2, and H-4 with C-3/C4a, indicating that the first prenyl group was attached to C-2. A strongly deshielded methylene group ($^{1H}\delta$ 3.93, $^{13C}\delta$ 33.9, H-16)



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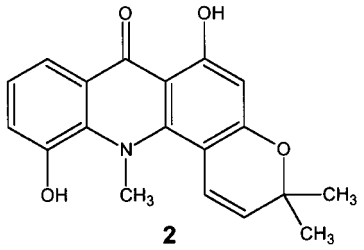

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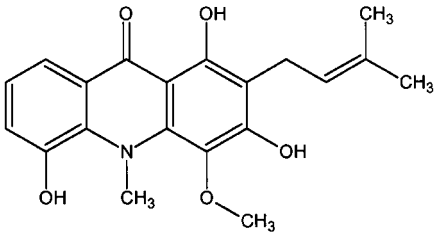

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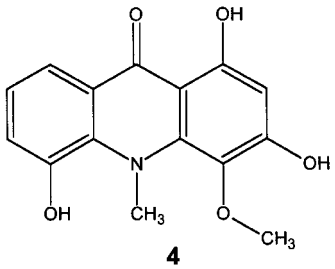

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Table 1. In Vitro Activity against *P. falciparum* Nigerian and FcM29 Strains[a]

compound	IC_{50} value against Nigerian strain in μM at 24 h	IC_{50} value against Nigerian strain in μM at 72 h	IC_{50} value against FcM29 strain in μM at 24 h	IC_{50} value against FcM29 strain in μM at 72 h
1,3,5-trihydroxy-2,8-bis (3-methylbut-2-enyl)-10-methyl-9-acridone (**1**)	5.3 ± 2.8	5.6 ± 3.8	7.9 ± 2.8	7.1 ± 2.0
5-hydroxynoracronycine (**2**)	11.1 ± 1.6	8.7 ± 0.9	10.2 ± 1.6	6.8 ± 0.9
1,3,5-trihydroxy-4-methoxy-2-(3-methylbut-2-enyl)-10-methyl-9-acridone (**3**)	2.5 ± 0.6	2.5 ± 1.1	2.0 ± 0.6	2.3 ± 0.6
1,3,5-trihydroxy-4-methoxy-10-methylacridone (**4**)	33.0 ± 10.3	20.1 ± 7.3	27.1 ± 5.1	20.5 ± 6.6
chloroquine diphosphate	0.07 ± 0.01	0.06 ± 0.01	0.45 ± 0.06	0.42 ± 0.08

[a] The mean IC_{50} values of the test compounds and standard drug ($n = 3$, $\pm\sigma$, n = number of tests performed in three series).

Table 2. Ratios of the in Vitro Cytotoxicity of the Test Compounds to Activity against *P. falciparum* Nigerian Strain[a]

compound	ED_{50} HeLa cells in μM at 24 h	ED_{50} HeLa cells in μM at 72 h	ratio of activity [ED_{50} Hela cells/ IC_{50} Nigerian strain] at 24 h	ratio of activity [ED_{50} Hela cells/ IC_{50} Nigerian strain] at 72 h
1,3,5-trihydroxy-2,8-bis (3-methylbut-2-enyl)-10-methyl-9-acridone (**1**)	47.2 ± 5.4	52.2 ± 4.4	9.0	9.4
5-hydroxynoracronycine (**2**)	85.5 ± 4.4	78.0 ± 9.2	7.7	9.0
1,3,5-trihydroxy-4-methoxy- 2-(3-methylbut-2-enyl)-10-methyl-9-acridone (**3**)	0.71 ± 0.11	5.30 ± 0.79	0.3	2.1
1,3,5-trihydroxy-4-methoxy-10-methylacridone (**4**)	16.8 ± 2.2	35.1 ± 8.2	0.5	1.8
chloroquine diphosphate	>100	>100	>1000	>1000

[a] $n = 3$, $\pm\sigma$ (n = number of tests performed in three series).

indicated that the second prenyl group was attached to the peri-position (C-8) of the 9-carbonyl group. The HMBC correlations of H-16 with C-8/C-8a/C-7, and H-6 with C-8, supported also this structure. Detailed analysis of HSQC, HMBC, COSY, and DEPT 135 spectra allowed the complete assignment of ^1H and ^{13}C signals and led to the assignment of structure **1** (1,3,5-trihydroxy-2,8-bis(3-methylbut-2-enyl)-10-methyl-9-acridone).

Antiviral and antitumor activities were recently described for a number of acridone alkaloids.[7−9] Earlier studies report antiplasmodial activity in vitro against *P. yoelii* and *P. falciparum* for acridone derivatives,[10,11] and the antimalarial activity of several dihydroacridinediones is currently under investigation.[12,13] To obtain preliminary information of a possible mode of action and prediction of cross resistance with chloroquine (CQ), the four isolated alkaloids were assessed for antiplasmodial activity on a Nigerian chloroquine-sensitive (CS) strain[14] and the chloroquine-resistant (CR) strain FcM29. As reported in Table 1, all the test compounds showed good antiplasmodial activity. Alkaloid **3** was found to be the most active pharmacophore, with no significantly different values against CS or CR *P. falciparum*, indicating the absence of in vitro cross resistance. However, as shown in Table 1, **3** was 5 times less active than chloroquine against the CR strain and 42 times less active than chloroquine against the CS strain. Alkaloids **2** and **4** showed higher antiplasmodial activity after 72 h than after 24 h of contact, while the activity of **1** and **3** was identical for the two times of contact. This may suggest that the activity of **2** and **4** takes place during, or just before, the metabolic pathways allowing the reinfection stage of the red cells by the merozoites of *P. falciparum*.

We have also evaluated the toxicity of the isolated alkaloids on Hela cells in order to determine the selectivity index (SI) of these potential chemotherapeutic agents. The results are summarized in Table 2. The ratio of cytotoxicity to biological activity for **1** and **2** suggests that antimalarial efficacy of these compounds is not due to in vitro cytotoxicity, this being, at 72 h for the CS strain, almost 10-fold greater than the IC_{50}, with values of 9.0 and 9.4, respectively. Under the same conditions, the selectivity of **3** and **4**, which both bear a methoxy group in position 4, is notably lower, with SI values of 2.1 and 1.8, respectively. Alkaloid **3**, which shows the highest antiplasmodial activity among the isolated acridones, is also the most cytotoxic in our assays, and its antiproliferative activity appears to be greater than those reported for other acridone alkaloids such as acronycine, 6-demethoxyacronycine, and their 11-amino derivatives.[15]

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on NMR Bruker DRX-300 at 300 MHz for ^1H NMR, $^1H-^1H$ COSY-gs, $^1H-^{13}C$ HSQC, and $^1H-^{13}C$ HMBC, and 75 MHz for ^{13}C NMR and ^{13}C DEPT 135 in DMSO-d_6 using standard Bruker microprograms. Chemical shifts are reported in ppm relative to DMSO-d_6 ($^{1H}\delta$ 2.49, $^{13C}\delta$ 39.5 ppm). In HMBC, 7.14 Hz is used as the long-range coupling constant between proton and carbon. UV spectral data were obtained on a Shimadzu UV-1205. FABMS and HRFABMS were taken on a JEOL-NS700 instrument.

Plant Material. Aerial parts and stem bark of *S. glutinosa* were collected near Cali (Colombia) in 1999 and identified by Lic. Robert Tulio Gonzalez. A voucher specimen (BW103) was deposited at the Herbarium of the Universidad del Valle, Cali (CUVC).

Extraction and Isolation. The powdered stem bark (400 g) was extracted exhaustively with MeOH at room temperature. After evaporation under reduced pressure, the residue (42 g) was fractionated by VCC over 1 kg of silica gel 60 (70−230 mesh, Merck) eluting with CH_2Cl_2 containing increasing amounts of MeOH. The 10−20% MeOH eluate was subjected to Si CC eluting with CH_2Cl_2 containing increasing amounts of MeOH, giving five fractions. Lupeol (30 mg) crystalized from fraction 1 (100% CH_2Cl_2) following adjunction of small quantities of MeOH. Further purification of fraction 2 (20% MeOH) was achieved by Si TLC using hexane−EtOAc (7:3) and gel permeation CC (Sephadex LH-20; 30 cm × 2 cm; MeOH; flow rate 1 mL/min), yielding **1** (6 mg), **2** (6 mg), **3** (10 mg), and **4** (5 mg).

1,3,5-Trihydroxy-2,8-bis(3-methylbut-2-enyl)-10-methyl-9-acridone (1): amorphous powder, UV (log ϵ) 262.5 (3.49), 288 (3.12), 320.5 (3.03), 409 (2.69) nm; ^1H NMR (DMSO-d_6,

300 MHz) δ 15.07 (1H, s, 1-OH), 7.07 (1H, d, J = 8.2 Hz, H-6), 6.84 (1H, J = 8.2 Hz, H-7), 6.28 (1H, s, H-4), 5.33 (1H, br tr, J = 6.87 Hz, H-17), 5.21 (1H, br tr, J = 6.83 Hz, H-12), 3.93 (2H, d, J = 6.96 Hz, H-16), 3.77 (3H, s, N-CH_3), 3.21 (2H, d, J = 6.57 Hz, H-11), 1.73 (3H, s, H-15), 1.68 (3H, s, H-20), 1.64 (3H, s, H-19), 1.61 (3H, s, H-14); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 182.0 (C-9), 163.5 (C-3), 161.0 (C-1), 146.0 (C-5), 145.0 (C-4a), 135.6 (C-10a), 132.6 (C-8), 130.0 (C-18), 129.8 (C-13), 124.9 (C-17), 124.0 (C-7), 123.2 (C-12), 120.8 (C-8a), 119.1 (C-6), 107.4 (C-2), 104.4 (C-9a), 90.4 (C-4), 42.0 (N-CH_3), 33.9 (C-16), 25.7 (sC-19), 25.5 (C-14), 17.9 (sC-20), 17.7 (C-15); MS m/z 394.2 $(M + 1)^+$; HRFABMS m/z 394.2010 $[M + 1]^+$ (calcd for $C_{24}H_{27}O_4N$: 394.4906).

Parasite Culture. The Nigerian strain of *P. falciparum*, chloroquine-sensitive with an inhibitory concentration (IC_{50}) of 67.8 ± 5.8 nM,[1,16] and the FcM29 strain, chloroquine-resistant (IC_{50} = 290.7 ± 34.9 nM), were cultured continuously according to Trager and Jensen.[17] The IC_{50} of chloroquine was checked every two months, and the cutoffs for chloroquine resistance were those reported by others.[18] Parasites were maintained in vitro in human red blood cells (O±), diluted to 1% hematocrit in RPMI 1640 medium supplemented with 25 mM Hepes and 30 mM $NaHCO_3$ and complemented with 5% human AB^+ serum. Parasite cultures were daily synchronized by gelatine flotation[17] and by 5% of sorbitol lysis.[19]

Cell Culture. Toxicity was evaluated on human fibroblasts (HeLa) cells. Cells were cultured under the same conditions as for *P. falciparum*, except for the 5% human serum, which was replaced by 5% fetal calf serum (Boehringer, Germany).

Test Compounds. The compounds were first diluted in DMSO (Merck) to 10 mg/mL. The mother solutions were then serially diluted in culture medium without serum. Final concentration ranged between 50 and 0.01 μg/mL for antiplasmodial activity testing and between 100 and 0.02 μg/mL for cytotoxicity (final concentration of DMSO never exceeded 1%).

Antiplasmodial Drug Assay. In vitro activity against *P. falciparum* was evaluated by using a modified radioactive micromethod.[20] Drug testing was performed three times in triplicate in 96-well culture plates (TPP, Switzerland) with cultures mostly at ring stage (synchronization interval: 16 h) at 0.5−1% parasitemia (hematocrit: 1%). For each test, parasite culture was incubated with drug for two times: 24 h and 72 h. Both strains of *P. falciparum* were used for this method, and parasite growth was estimated by [^{3}H]-hypoxanthine incorporation.[21] IC_{50} values were determined graphically in concentration versus percent inhibition curves.

Cytotoxicity Assay. For the determination of the in vitro toxicity of the test compounds, cells were distributed after trypsinization (Trypsin-EDTA, Gibco BRL, Paisley, Scotland) in 96-well plates at 2×10^4 cells/well in 100 μL, then 100 μL of culture medium containing the test compounds was added. Drug testing was performed three times in triplicate. Cell growth was determined by [^{3}H]-hypoxanthine incorporation, with and without the test compounds, after 24 and 72 h incubation exactly as for the *P. falciparum* contact period.[22]

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