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Antiproliferative and antimalarial anthraquinones of *Scutia myrtina* from the Madagascar forest¹

Yanpeng Hou¹, Shugeng Cao¹, Peggy J. Brodie¹, Martin W. Callmender², Fidisoa Ratovoson², Etienne Rakotobe³, Vincent E. Rasamison³, Michel Ratsimbason³, John N. Alumasa⁴, Paul D. Roepe⁴, and David G. I. Kingston¹

¹Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA

²Missouri Botanical Garden, BP 3391, Antananarivo 101, Madagascar

³Centre National d'Application des Recherches Pharmaceutiques, B.P. 702, Antananarivo 101, Madagascar

⁴Department of Chemistry, Georgetown University, 37th and O Streets, NW Washington, D.C. 20057, USA

Abstract

Bioassay-guided fractionation of an ethanol extract of a Madagascar collection of the bark of *Scutia myrtina* led to the isolation of three new anthrone-anthraquinones, scutianthraquinones A, B and C (**1–3**), one new bisanthrone-anthraquinone, scutianthraquinone D (**4**), and the known anthraquinone, aloesaponarin I (**5**). The structures of all compounds were determined using a combination of 1D and 2D NMR experiments, including COSY, HSQC, HMBC, and ROESY sequences, and mass spectrometry. All the isolated compounds were tested against the A2780 human ovarian cancer cell line for antiproliferative activities, and against the chloroquine-resistant *Plasmodium falciparum* strains Dd2 and FCM29 for antiplasmodial activities. Compounds **1**, **2** and **4** showed weak antiproliferative activities against the A2780 ovarian cancer cell line, while compounds **1–4** exhibited moderate antiplasmodial activities against *P. falciparum* Dd2 and compounds **1**, **2**, and **4** exhibited moderate antiplasmodial activities against *P. falciparum* FCM29

1. Introduction

In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Groups (ICBG) program, we obtained an extract from the bark of *Scutia myrtina* collected in Madagascar. The roots of *Scutia myrtina* have been investigated previously, and afforded two perylenequinones with in vitro anthelmintic activity but no in vivo activity.² In addition, several cyclopeptide alkaloids have been isolated from other *Scutia* species,^{3–6} some of which showed moderate antimicrobial activity.³ In our research, the extract was selected for bioassay-guided fractionation on the basis of its antiproliferative activity against the A2780 human ovarian cancer cell line, with an

Correspondence to: David G. I. Kingston.

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IC₅₀ value of 6 µg/mL. The crude extract afforded three new anthrone-anthraquinones (**1-3**), a new bisanthrone-anthraquinone (**4**) and the known anthraquinone aloesaponarin I (**5**)⁷ after solvent partition and reversed-phase C18 HPLC. Herein we report the structural elucidation of the new compounds and their bioactivities against the A2780 human ovarian cancer cell line and two strains of the malaria parasite *Plasmodium falciparum*.

2. Results and discussion

Scutianthraquinone A (**1**, Figure 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as C₃₉H₃₂O₁₃ on the basis of its molecular ion peak in its HRFAB mass spectrum. Its UV-VIS spectra showed characteristic absorptions of anthraquinones at 248, 272, 310 and 475 nm.⁸ The ¹H NMR spectrum of **1** (Table 1) in CD₃OD showed signals for an AB system at δ_H 8.64 (d, *J* = 7.8 Hz, H-3) and δ_H 7.86 (d, *J* = 7.8 Hz, H-4), an ABC system at δ_H 6.86 (br, d, *J* = 7.7 Hz, H-2'), δ_H 7.33 (t, *J* = 7.7 Hz, H-3') and δ_H 6.71 (br, d, *J* = 7.7 Hz, H-4'), two aromatic proton singlets at δ_H 7.52 (s, H-5) and δ_H 6.66 (s, H-5'), two methoxyl groups at δ_H 3.89 (s, 7-COOCH₃) and δ_H 3.88 (s, 7'-COOCH₃), and two methyl groups at δ_H 2.49 (s, C-8-CH₃) and δ_H 2.77 (s, C-8'-CH₃). The ¹H NMR spectrum in DMSO-*d*₆ also showed resonances for four hydroxyl protons at δ_H 13.68, 13.24, 11.80 and 10.99, indicating that each was intramolecularly hydrogen bonded. In the ¹³C NMR spectrum six carbonyl carbons, 24 aromatic carbons, one oxygenated quaternary carbon, two methoxyl carbons, one methine carbon, one methylene carbon and four methyl carbons were identified.

On the basis of its UV, ¹H NMR, and ¹³C NMR spectra and its molecular formula, the structure of **1** was assigned as an anthrone-substituted anthraquinone.

The complete ¹H NMR and ¹³C NMR assignments and connectivities of **1** were determined from a combination of COSY, HSQC, HMBC and ROESY data. The COSY spectrum showed correlations that confirmed the connectivity of H-3 and H-4, and of H-2', H-3' and H-4'. In the HMBC spectrum, the correlations of H-4 and H-5 to C-10 (δ_C 183.6) and H-4' and H-5' to C-10' (δ_C 78.2) indicated the positions of these protons. The position of CH₃-8 was established by three-bond HMBC correlations from the protons of CH₃-8 and H-5 to C-7 and C-8a (δ_C 131.8, 123.5) along with a two-bond correlation from the protons of CH₃-8 to C-8 (δ_C 143.5). The position of CH₃-8' was established by three-bond HMBC correlations from protons of CH₃-8' and H-5' to C-7' and C-8'a (δ_C 126.6, 124.1) along with two-bond correlations from protons of CH₃-8' to C-8' (δ_C 141.9). The presence of two carbomethoxy groups (COOCH₃-7, COOCH₃-7') was indicated by HMBC correlations from their methyl protons to the corresponding carbonyl carbons (COOCH₃-7, 7', δ_C 170.6, 169.9), and their positions were established by ROESY correlations (Fig. 2) and by comparison of ¹³C NMR data for C-5/6/7/8/8a/10a and C-5'/6'/7'/8'/8'a/10'a of **1** and the corresponding carbons of the known anthraquinone aloesaponarin I (**5**).⁷ A ROESY correlation from the protons of 7'-COOCH₃ to those of CH₃-8' unambiguously located the carbomethoxy group at C-7' instead of C-6'. A corresponding ROESY correlation from the protons of COOCH₃-7 to CH₃-8 was not observed, but the almost identical chemical shifts between C-5/6/7/8/8a/10a and C-5'/6'/7'/8'/8'a/10'a in the ¹³C NMR of **1** indicated that COOCH₃-7 must reside on C-7 instead of C-6. Finally, the chemical shifts of C-5/6/7/8/8a/10a showed the same pattern as those of C-5/6/7/8/8a/10a of **5**, offering further confirmation of the assigned positions of the carbomethoxy groups.

The anthrone and anthraquinone moieties were connected through the bond between C-2 and C-10', as evidenced by the key HMBC correlation of H-3 to C-10' (δ_C 78.2) (Figure 2). The presence of a 2-methylbutanoyl group was established by COSY correlations between a methine proton (δ_H 2.53, m) and methyl (δ_H 1.15/1.12, d, *J* = 7.2 Hz) and methylene protons

(δ_{H} 1.69, m; δ_{H} 1.53, m). The methylene protons were part of an ethyl group, as shown by the COSY correlations to methyl protons at δ_{H} 0.85 (t, J = 7.4 Hz) (Figure 1).

Supporting evidence for the 2-methylbutanoyl group was provided by HMBC correlations from its methylene protons (δ_{H} 1.69, m; δ_{H} 1.53, m) and methyl protons (δ_{H} 1.15/1.12, d, J = 7.2 Hz) to a carbonyl group (δ_{C} 174.9) (Figure 2). The group was assigned to the C-10' position by ROESY correlations from CH₃-2'' (δ_{H} 1.15/1.12, d, J = 7.2 Hz) to H-3 and H-4' and from H₃-4'' (δ_{H} 0.85, t, J = 7.4 Hz) to H-4 and H-5' (Figure 3). The negative ion ESI-MS/MS of **1** gave a series of fragment ions which were entirely consistent with the assigned structure. The structure of **1**, except for the absolute configurations of C-10' and the 2-methylbutanoyl group, was thus established.

Analysis of the ¹H and ¹³C NMR spectra of **1** indicated the existence of two atropisomers. The signals for H-3' in its ¹H NMR spectrum in CD₃OD appeared as two overlapping triplets, while the signal for H-5' appeared as two singlets; each signal integrated for only one proton. In addition, the signal of CH₃-2'' of the 2-methylbutanoyl group was split into two overlapping doublets, and that of H₃-4'' appeared as two overlapping triplets. The splittings of the signals for C-8, C-4', C-5, and the carbons of the 2-methylbutanoyl group were also observed in the ¹³C NMR spectrum. A series of ¹H NMR spectra of **1** were obtained in DMSO-*d*₆ at a series of elevated temperatures, and the split signals of the above protons either merged completely (H-3' and H-5') or partially (the protons of the two methyl groups in the 2-methylbutanoyl group) as the temperature increased. An example is shown in Table 2. The atropisomerism of **1** presumably arises because the rotation of the C-2–C-10' bond is restricted by steric effects.

Scutianthraquinone B (**2**, Figure 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as C₃₈H₃₀O₁₃, differing from that of **1** by a CH₂ group, on the basis of its [M-H] peak in its negative ion HRESI mass spectrum. The UV-VIS spectra showed characteristic features of anthraquinones at 248, 272, 310 and 475 nm. The ¹H NMR spectrum of **2** was almost identical to that of **1**, except that the signals from the C-10' substituent lacked signals for one methylene group compared with **1** (Table 1). This observation indicated that an isobutanoyl group in **2** had replaced the 2-methylbutanoyl group in **1**. HMBC correlations of the H-3a'' and H-3b'' methyl protons (δ_{H} 1.17/1.15, d, J = 7.2 Hz) to C-1'' (δ_{C} 174.0) and C-2'' (δ_{C} 35.0) and TOCSY correlations between the H₃-3a''/H₃-3b'' methyl protons and H-2'' (δ_{H} 2.68, m) were in agreement with the above assignment. The other HMBC and TOCSY correlations of **2** showed the same patterns as those of **1**, and confirmed the similarity between **2** and **1**. An attempt to determine if the isobutanoyl group was located at C-10' by ROESY correlations by the same strategy applied to **1** was not successful, due to the small quantity of **2** and a corresponding weak signal. The substituent was thus assigned to C-10' based on the close similarity of the ¹H NMR chemical shifts of **1** and **2**. If the isobutanoyl group were located at any other position significant differences in these shifts would be expected, as observed for the monomeric anthraquinone aloesaponarin I and its acetylated form.⁹ In addition four intramolecularly hydrogen bonded hydroxyl protons were observed at δ_{H} 14.89, 13.25, 11.92 and 10.79 in the ¹H NMR spectrum of **2** in DMSO-*d*₆. The LC-ESI-MS/MS profile of **2** was essentially identical to that of **1**, after allowing for the molecular weight differences, and thus also supported the proposed structure of **2**. The proposed fragmentation pattern is shown in Figure S1. Based on the arguments above, the structure of **2** was assigned as shown in Figure 1.

Scutianthraquinone C (**3**, Figure 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as C₃₄H₂₄O₁₂ on the basis of its molecular ion peak in its HRFAB mass spectrum. The UV-VIS spectra showed characteristic features of anthraquinones at 248, 272, 310 and 475 nm. The ¹H NMR spectrum of **3** was almost identical to that of **1**

except for the absence of signals for the 2-methylbutanoyl group in **1**, indicating the presence of a free hydroxyl group at C-10'. The HMBC and COSY spectra and LC-ESI-MS/MS profile (see proposed fragmentation pattern in Figure S1) of **3** supported the above deduction. Thus, the structure of **3** was established as shown in Figure 1.

Scutianthraquinone D (**4**, Figure 4) was obtained as a light-brown amorphous solid. Its molecular formula was established as $C_{61}H_{52}O_{20}$ on the basis of its [M-H] peak in its negative ion HRESI mass spectrum. The UV-VIS spectra showed characteristic features of anthraquinones at 248, 272, 310, 353 and 475 nm. In the 1H NMR spectrum of **4** in CD_3OD , two AB systems (δ_H 8.67, d, $J = 7.8$ Hz, H-3 and δ_H 7.95, d, $J = 7.8$ Hz, H-4, and δ_H 8.32, d, $J = 8.5$ Hz, H-3' and δ_H 6.92, d, $J = 8.5$ Hz, H-4') and one ABX system (δ_H 6.85, br, d, $J = 8.1$ Hz, H-2'', δ_H 7.30, t, $J = 8.1$ Hz, H-3'' and δ_H 6.62, br, d, $J = 8.1$ Hz, H-4'') were observed, and three aromatic proton singlets (δ_H 7.53, H-5 and δ_H 6.60/6.59, 6.58/6.57, H-5', H-5'') also appeared. Singlets for three methoxyl protons (δ_H 3.90, 3.89, 3.84) and three methyl protons (δ_H 2.53, 2.60, 2.78) were also observed. In the 1H NMR spectrum of **4** in $DMSO-d_6$, signals for six intramolecularly hydrogen bonded hydroxyl protons (δ_H 14.92, 13.66, 13.23, 11.91, 10.77 and 9.71) were also observed. The COSY spectrum of **4** confirmed the aforementioned AB and ABC systems, and COSY correlations of the protons from two 2-methylbutanoyl groups also were observed. Careful comparison of 1H NMR and COSY data of **4** to those of **1** indicated the presence of an additional monomeric anthrone unit in **4**, indicating a trimeric structure. The existence of six intramolecularly hydrogen bonded hydroxyl protons and comparison of the chemical shifts and coupling patterns in the 1H NMR of **4** to those of **1** indicated that the two 2-methylbutanoyl groups must be located at C-10 and C-10', and established the structure of **4** as the bisanthrone-anthraquinone shown in Figure 1. The LC-ESI-MS/MS spectrum of **4** fully supported the proposed structure, and the proposed fragmentation pathways of **4** are shown in Figure S2.

Only a few anthrone-anthraquinones with the two moieties connected by a bond between the C-2 position of the anthraquinone unit and C-10' of the anthrone unit have been reported,¹⁰⁻¹⁶ and compounds **1-3** are novel examples of this relatively rare structural type. Compound **4** is the first reported bisanthrone-anthraquinone isolated from Nature.

The structure of aloesaponarin I (**5**, Figure 5) was assigned by comparison of its spectroscopic data with literature data.⁷

The isolated compounds were tested against the A2780 human ovarian cancer cell line and were also tested against chloroquine-resistant strains of *Plasmodium falciparum* Dd2 and FCM29. The results are shown in Table 3. Compounds **1-5** exhibited weak antiproliferative activities against the A2780 human ovarian cancer cell line, with the trimer **4** being slightly more active than dimers **1**, **2** and **3**, and the monomer **5** showed the weakest activity. Compounds **1-5** showed moderate antiparasmodial activities against the chloroquine-resistant *P. falciparum* Dd2, with IC_{50} values in the range of 1-6 μM ; the dimers **1-3** showed slightly better activities than the trimer **4**, and the monomer **5** displayed the least activity. The dimers **1** and **2** with C-10' substituents exhibited slightly better activities than the dimer **3** with a free hydroxyl group at C-10'. With respect to the activities against *P. falciparum* FCM29, compounds **1**, **2**, and **4** showed moderate activities with IC_{50} values from 1.2 to 5.6 μM compared to chloroquine ($IC_{50} = 0.41 \mu M$). The selectivities of compounds **1-5** for antiparasmodial as opposed to antiproliferative activities, as determined by the quotient of antiproliferative activity and antiparasmodial activity, vary between 0.8 and 6 (Table 3).

These selectivities are lower than would be desirable for future drug development, and this fact, coupled with the chemical complexity of the compounds, makes them challenging compounds to develop. Some related anthrone-anthraquinones had selectivities of up to 400

between the chloroquine-sensitive 3D7 strain of *P. falciparum* and the KB cell line,¹⁷ so it is possible that compounds of this class might ultimately be developable as antimalarial agents. This conclusion is supported by the fact that some phenylanthraquinones, including monomeric^{18,19} and dimeric examples,²⁰ have promising antimalarial bioactivities.

3. Experimental section

3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were obtained on a Shimadzu UV-1201 spectrophotometer. NMR spectra were obtained on Bruker Avance 600, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRFAB mass spectra were obtained on a JEOL-JMS-HX-110 instrument. HRESI mass spectra were obtained on an Agilent 6220 TOF LC/MS. LC-ESIMS was performed on Agilent 1100 and Thermo TSQ Quantum instruments. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative column (250 $\times$ 10 mm). The HPLC instrument employed a Shimadzu SPD-M10A diode array detector.

3.2. Antiproliferative Bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.²¹ The A2780 cell line is a drug sensitive ovarian cancer cell line.²²

3.3. Antimalarial Bioassay

Antiplasmodial assays with the chloroquine-resistant strain *Plasmodium falciparum* FCM29 were performed at the Centre National d'Application des Recherches Pharmaceutiques. Assays with the chloroquine-resistant *Plasmodium falciparum* Dd2 were performed at Georgetown University. Both assays used the previously reported Cybr green method.²³

3.4. Plant material collection and extraction

Bark of the climbing shrub *Scutia myrtina* (Burm. f.) Kurz (Rhamnaceae) was collected in the Montagne des Français region, a dry forest on limestone, Antsiranana, Madagascar, at an elevation of 280 m, at 12.24.41 S, 49.22.17 E, on February 14, 2005. Its assigned collection number is Randrianasolo.S (SSR) et al. 517. The collection was made from a woody liana, with one pair of spines in each node, green fruit becoming black when mature, brown seeds, growing on slope. Its vernacular name is *Roiavotra*. *Scutia myrtina* has a large distribution from Africa to south east Asia. This species is somewhat variable but the variation is chaotic and nearly coextensive throughout the vast range, so that the recognition of infraspecific taxa is not practicable. Voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); the Missouri Botanical Garden, St. Louis, Missouri (MO); and the Muséum National d'Histoires Naturelles, Paris, France (P).

The dried and powdered bark of *Scutia myrtina* (254 g) was extracted at room temperature with EtOH (1.2 L) for 24 h. After filtration, the solvent was evaporated to dryness under reduced pressure, affording a crude ethanolic extract (7.86 g).

3.5. Extract fractionation

A total of 2.5 g of extract was supplied to Virginia Polytechnic Institute and State University, and this had an IC₅₀ value of 12 μ g/mL against A2780 cells. This extract (2.0 g) was suspended in aqueous MeOH (90%MeOH-H₂O, 60 mL) and extracted with hexanes (3 $\times$ 60 mL). The

aqueous layer was then diluted to 60% MeOH (v/v) with H₂O and extracted with CH₂Cl₂ (3 × 90 mL). The CH₂Cl₂ extract (82.2 mg) was found to be the most active against A2780 cells (IC₅₀ = 6 µg/mL) and was separated via semipreparative HPLC over a C18 column using MeOH-H₂O (75:25) to afford 9 fractions (I-IX). Fractions II, III, VI, VII and VIII afforded aloesaponarin I (**5**, 1.2 mg, *t_R* 19.6 min), scutianthraquinone C (**3**, 1.6 mg, *t_R* 21.4 min), scutianthraquinone B (**2**, 1.0 mg, *t_R* 31.6 min), scutianthraquinone D (**4**, 2.0 mg, *t_R* 34.6 min) and scutianthraquinone A (**1**, 2.9 mg, *t_R* 38.5 min), respectively.

3.6. Scutianthraquinone A (**1**)

Light-brown amorphous solid; $[\alpha]_D^{25} +60.8$ (*c* 0.06, CHCl₃); UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 203 (4.35), 248 (4.09), 272 (4.01), 310 (4.03), 351 sh (3.83) and 475 sh (3.36); HRFABMS *m/z*: 708.1790 [M][−] (Calcd for C₃₉H₃₂O₁₃: 708.1843); LC-ESIMS *m/z* (rel. int.): [M-H][−] 707 (1), 675 (21), 643 (1), 605 (4), 577 (100), 573 (14), 545 (40), 541 (2), 517 (3); ¹H NMR: see Table 1; ¹³C NMR (CD₃OD): 192.2 (C-9/C-9'), 190.9 (C-9/C-9'), 183.6 (C-10), 174.9 (C-10'-OCOCH(CH₃)CH₂CH₃), 170.6 (C-7-COOCH₃), 169.9 (C-7'-COOCH₃), 163.2 (C-1'), 159.8 (C-1), 149.1 (C-6), 149.1 (C-6'), 143.6 (C-4'a), 143.5 (C-8), 141.9 (C-8'), 140.6 (C-2), 138.6 (C-10a/c-10'a), 136.0 (C-10a/C-10'a), 135.9 (C-3'), 133.9 (C-4a), 131.8 (C-7/8a), 131.7 (C-3), 126.6 (C-7'/8a'), 124.1 (C-7'/8a'), 123.5 (C-7/8a), 119.2 (C-4), 119.1 (C-9'a), 118.6 (C-9a), 118.4 (C-2'), 118.1/118.0 (C-4'), 113.9 (C-5), 112.7/112.6 (C-5'), 78.2 (C-10'), 53.0 (C-7-COOCH₃), 53.0 (C-7'-COOCH₃), 43.0 (C-10'-OCOCH(CH₃)CH₂CH₃), 28.0/27.9 (C-10'-OCOCH(CH₃)CH₂CH₃), 21.2 (8'-CH₃), 20.6 (8-CH₃), 17.0/16.9 (C-10'-OCOCH(CH₃)CH₂CH₃), 12.0 (C-10'-OCOCH(CH₃)CH₂CH₃).

3.7. Scutianthraquinone B (**2**)

Light-brown amorphous solid; $[\alpha]_D^{25} +134.8$ (*c* 0.04, CHCl₃); UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 203 (4.13), 248 (3.85), 272 (3.78), 310 (3.74), 353 sh (3.54) and 475 (3.08); HRESIMS *m/z*: 693.1614 [M-H][−] (Calcd for C₃₈H₃₀O₁₃: 693.1608); LC-ESIMS *m/z* (rel. int.): 693 (1) [M-H][−], 661 (48), 629 (4), 605 (12), 577 (100), 573 (22), 545 (80), 541 (2), 517 (8); ¹H NMR: see Table 1.

3.8. Scutianthraquinone C (**3**)

Light-brown amorphous solid; $[\alpha]_D^{25} +122.2$ (*c* 0.04, MeOH); UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 203 (4.24), 248 (3.98), 272 (3.92), 310 (3.90), and 475 sh (3.29); HRFABMS *m/z* 624.1296 [M][−] (Calcd for C₃₄H₂₄O₁₂: 624.1268); LCESIMS *m/z* (rel. int.): 623 (12) [M-H][−], 605 (4), 591 (100), 577 (88), 573 (28), 545 (84), 541 (64), 517 (12), 513 (10), 487 (8), 459 (4), 311 (5); ¹H NMR: see Table 1.

3.9. Scutianthraquinone D (**4**)

Light-brown amorphous solid; $[\alpha]_D^{25} -34.7$ (*c* 0.04, CHCl₃); UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 203 (4.64), 248 (4.40), 272 (4.34), 310 (4.25), 353 (4.12) and 475 sh (3.51); HRESIMS *m/z*: 1103.2977 [M-H][−] (Calcd for C₆₁H₅₂O₂₀: 1103.2979); LC-ESIMS *m/z* (rel. int.): 1103 (20) [M-H][−], 1071 (1), 1001 (6), 899 (40), 871 (100), 867 (14), 839 (4), 811(1); ¹H NMR: see Table 1.

3.10. Aloesaponarin I (**5**)

Light-brown amorphous solid; UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 215 (4.00), 270 (3.87), 280 (3.86), 307 sh (3.53), 408 (3.27), 430 (3.26) and 475 sh (2.98); HRFABMS *m/z*: 312.0609 [M][−] (Calcd for C₁₇H₁₂O₆: 312.0634); ¹H NMR (400 MHz, CDCl₃) δ 7.80 (1H, s, H-5), 7.78 (1H, br, d, *J* = 8.0 Hz, H-4), 7.63 (1H, t, *J* = 8.0 Hz, H-3), 7.32 (1H, br, d, *J* = 8.0 Hz, H-2), 4.07 (3H, s, 7-COOCH₃), 2.98 (3H, s, 8-CH₃); ¹H NMR (500 MHz, CD₃OD) δ 7.70 (1H, br, d, *J* = 7.8 Hz, H-4), 7.65 (1H, t, *J* = 7.8 Hz, H-3), 7.61 (1H, s, H-5), 7.28 (1H, br, d, *J* = 7.8 Hz, H-2), 3.94

(3H, s, 7-COOCH₃), 2.69 (3H, s, 8-CH₃); ¹³C NMR (500 MHz, CD₃OD) δ 191.1 (C-9), 184.0 (C-10), 169.9 (7-COOCH₃), 163.7 (C-1), 161.7 (C-6), 143.3 (C-8), 138.8 (C-10a), 137.0 (C-3), 134.3 (C-4a), 131.7 (C-8a/7), 125.7 (C-2), 124.0 (C-7/8a), 119.7 (C-4), 118.4 (C-9a), 113.7 (C-5), 53.2 (7-COOCH₃), 20.6 (8-CH₃).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and notes

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	1"	2"	3"	4"
1	$R = \text{COCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$			
2	$R = \text{COCH}(\text{CH}_3)_2$			
3	$R = \text{H}$			

Figure 1.
Structures of compounds **1** – **3**.

Figure 2.
Key COSY (bold) and HMBC (arrows) correlations of **1**

Figure 3.
Key ROESY correlations of **3**

Figure 4.
Structure of compound 4.

Figure 5.
Structure of compound 5.

Table 1

¹H NMR Data of Compounds **1-4**^c

no.	1 ^a	2 ^a	3 ^a	4 ^a
3	8.64 d (7.8)	8.65 d (8.1)	8.66 d (8.0)	8.67 d (7.8)
4	7.86 d (7.8)	7.92 d (8.1)	7.89 d (8.0)	7.95 d (7.8)
5	7.52 s	7.54 s	7.57 s	7.53 s
7-COOCH ₃	3.89 s	3.89 s	3.89 s	3.90 s ^e
8-CH ₃	2.49 s	2.54 s	2.55 s	2.53 s ^f
2'	6.86 br d (7.7)	6.86 br d (8.3)	6.83 br d (8.0)	
3'	7.33/7.33 t (7.7, 7.7) ^d	7.33 t (8.3, 8.3)	7.33 t (8.0, 8.0)	8.32/8.32 d (8.5) ^d
4'	6.71 br d (7.7)	6.71 br d (8.3)	6.76 d (8.0)	6.92 d (8.5)
5'	6.67/6.66 s ^d	6.65 s	6.75 s	6.60/6.59 s ^d
7'-COOCH ₃	3.88 s	3.88 s	3.89 s	3.84 s ^e
8'-CH	2.77 s	2.76 s	2.74 s	2.60 s ^f
10'-OCOCH(CH ₃)CH ₂ CH ₃	2.53 m			2.41 m ^g
10'-OCOCH(CH ₃)CH ₂ CH ₃	1.69 m 1.53 m			1.62 m ^g
10'-OCOCH(CH ₃)CH ₂ CH ₃	1.15/1.12 d (7.2) ^d			1.05/1.03 d (6.8) ^{d,g}
10'-OCOCH(CH ₃)CH ₂ CH ₃	0.85/0.85 t (7.4) ^d			0.85/0.84 t (7.1) ^{d,g}
10'-OCOCH(CH ₃) ₂		2.68 m		
10'-OCOCH(CH ₃) ₂		1.17/1.15 d (7.2) ^d		
2''				6.85 br d (8.1)
3''				7.30 t (8.1)
4''				6.62 br d (8.1)
5''				6.58/6.57 s ^d
7''-COOCH ₃				3.89 s ^e
8''-CH ₃				2.78 s
10''-OCOCH(CH ₃)CH ₂ CH ₃				2.58 m ^g
10''-OCOCH(CH ₃)CH ₂ CH ₃				1.69 m ^g
10''-OCOCH(CH ₃)CH ₂ CH ₃				1.17/1.16 d (6.3) ^{d,g}
10''-OCOCH(CH ₃)CH ₂ CH ₃				0.76 t (7.1) ^g
OH ^b	13.68 br s 13.24 s 11.80 br s 10.99 br s	14.89, 13.25, 11.92 10.79 (all br s)	13.23, 11.93, 11.73, 10.90 (all br s)	14.92, 13.66, 13.23, 11.91, 10.77, 9.71 (all br s)

^a in CD₃OD.^b in DMSO-*d*₆.^c δ (ppm) 500 & 600 MHz, 25°C.^d splits of signals due to.^{e,f} resonances may be interchanged.

^g resonances for 10'-OCO(CH₃)CHCH₂CH₃ and 10''-OCO(CH₃)CHCH₂CH₃ may be interchanged.

Table 2

Dependence of the separation between the two resonances of the 2''-CH₃ group with temperature in the variable temperature ¹H NMR spectrum of **1**.^a

Temperature (°C)	25	50	80	100	120
Δδ (Hz)	20.0	16.2	12.4	10.2	8.5

^a¹H NMR data was obtained in DMSO-*d*₆ using a 600 MHz NMR spectrometer.

Table 3

IC₅₀ (μM) values of **1-5** against Human A2780^a and *Plasmodium falciparum* strains FCM29^b and Dd2^c

Compound	A2780 IC ₅₀ (μM)	<i>P. falciparum</i> Dd2 IC ₅₀ (μM)	Selectivity Index (Dd2 vs A2780)	<i>P. falciparum</i> FCM29 IC ₅₀ (μM)	Selectivity Index (FCM29 vs A2780)
CQ	NT	0.147		0.41	
Quinine	NT	NT		0.22	
1	7.6 ± 0.8	1.23	6.1	1.2	6.3
2	5.8 ± 2.5	1.14	5.1	5.4	1.1
3	>16	3.14	>5	15.4	>1
4	4.3 ± 0.9	3.68	1.2	5.6	0.8
5	>32	5.58	>5.7	77.6	NM

^aPaclitaxel (IC₅₀ 0.014 μM) was the positive control.²¹