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Antiproliferative and antimalarial anthraquinones of *Scutia myrtina* from the Madagascar forest ☆

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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, TOCSY, 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 IC_{50} value of 12 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**, Fig. 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as $C_{39}H_{32}O_{13}$ on the basis of its molecular ion peak in its negative ion HRFAB mass spectrum. Its UV–vis spectra showed characteristic absorptions of anthraquinones at 248, 272, 310 and 475 nm.[8]

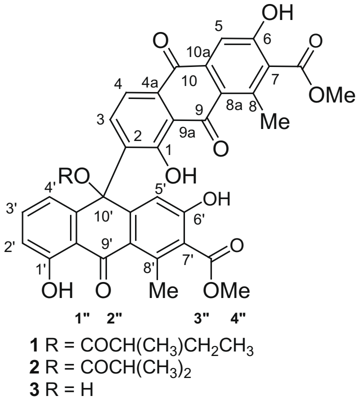

Figure 1. Structures of compounds **1**–**3**.

☆ See Ref. 1.

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The ^{1}H NMR spectrum of **1** (Table 1) in CD_3OD 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 7.330/7.326 (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 6.67/6.66 (s, H-5′), two methoxyl groups at δ_H 3.89 (s, $COOCH_3$-7) and δ_H 3.88 (s, $COOCH_3$-7′), and two methyl groups at δ_H 2.49 (s, CH_3-8) and δ_H 2.77 (s, CH_3-8′). The ^{1}H 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 ^{13}C 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-vis, ^{1}H NMR, and ^{13}C NMR spectra and its molecular formula, the structure of **1** was assigned as an anthrone–substituted anthraquinone.

The complete ^{1}H NMR and ^{13}C 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 CH_3-8 was established by three-bond HMBC correlations from the protons of CH_3-8 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 CH_3-8 to C-8 (δ_C 143.5). The position of CH_3-8′ was established by three-bond HMBC correlations from protons of CH_3-8′ and H-5′ to C-7′ and C-8′a (δ_C 126.6, 124.1) along with two-bond correlations from protons of CH_3-8′ to C-8′ (δ_C 141.9). The presence of two carbomethoxy groups ($COOCH_3$-7, $COOCH_3$-7′) was indicated by HMBC correlations from their methyl protons to the corresponding carbonyl carbons ($COOCH_3$-7, 7′, δ_C 170.6, 169.9), and their positions were established by ROESY correlations (Fig. 2) and by comparison of ^{13}C 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′-$COOCH_3$ to those of CH_3-8′ unambiguously located the carbomethoxy group at C-7′ instead of C-6′. A corresponding ROESY correlation from the protons of $COOCH_3$-7 to CH_3-8 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 ^{13}C NMR of **1** indicated that $COOCH_3$-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) (Fig. 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 0.85/0.83 (t, J = 7.4 Hz) (Fig. 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) (Fig. 2). The group was assigned to

Table 1
^{1}H 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′	7.330/7.326 t (7.7, 7.7)[d]	7.33 t (8.3, 8.3)	7.33 t (8.0, 8.0)	8.33/8.32 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_3	2.77 s	2.76 s	2.74 s	2.60 s[f]
10′-$OCOCH(CH_3)CH_2CH_3$	2.53 m			2.41 m[g]
10′-$OCOCH(CH_3)CH_2CH_3$	1.69 m			1.62 m[g]
	1.53 m			
10′-$OCOCH(CH_3)CH_2CH_3$	1.15/1.12 d (7.2)[d]			1.05/1.03 d (6.8)[d,g]
10′-$OCOCH(CH_3)CH_2CH_3$	0.85/0.83 t (7.4)[d]			0.85/0.84 t (7.1)[d,g]
10′-$OCOCH(CH_3)_2$		2.68m		
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[f]
10″-$OCOCH(CH_3)CH_2CH_3$				2.58 m[g]
10″-$OCOCH(CH_3)CH_2CH_3$				1.69 m[g]
10″-$OCOCH(CH_3)CH_2CH_3$				1.17/1.16 d (6.3)[d,g]
10″-$OCOCH(CH_3)CH_2CH_3$				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 CD_3OD.
[b] In DMSO-d_6.
[c] δ (ppm) 500 and 600 MHz, 25 °C.
[d] Doubling of signals due to atropisomers.
[e,f] Resonances may be interchanged.
[g] Resonances for 10′-$OCO(CH_3)CHCH_2CH_3$ and 10″-$OCO(CH_3)CHCH_2CH_3$ may be interchanged.

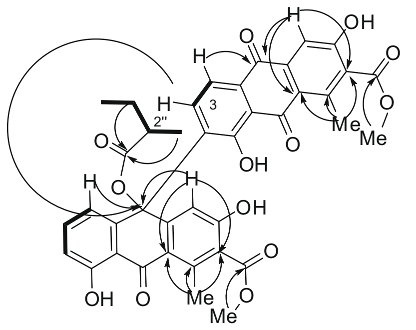


Figure 2. Key COSY (bold) and HMBC (arrows) correlations of **1**.

the C-10′ position by ROESY correlations from CH_3-2″ (δ_H 1.15/1.12, d, $J = 7.2$ Hz) to H-3 and H-4′ and from H_3-4″ (δ_H 0.85/0.83, t, $J = 7.4$ Hz) to H-4 and H-5′ (Fig. 3). The negative ion LC–ESI–MS/MS of **1** gave a series of fragment ions which were entirely consistent with the assigned structure. The proposed fragmentation pattern is shown in Figure S1. 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 ^{13}C NMR spectra of **1** indicated the existence of two atropisomers. The signals for H-3′ in its ^1H NMR spectrum in CD_3OD 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 CH_3-2″ of the 2-methylbutanoyl group was split into two overlapping doublets, and that of H_3-4″ 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 ^{13}C 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.

Table 2
Dependence of the separation between the two resonances of the 2″-CH_3 group with temperature in the variable temperature ^1H NMR spectra of **1**[a]

Temperature (°C)	25	50	80	100	120
$\Delta\delta$ (Hz)	20.0	16.2	12.4	10.2	8.5

[a] ^1H NMR data were obtained in DMSO-d_6 using a 600 MHz NMR spectrometer.

Scutianthraquinone B (**2**, Fig. 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as $C_{38}H_{30}O_{13}$, differing from that of **1** by a CH_2 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_3-3a″ and H_3-3b″ 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 H_3-3a″/H_3-3b″ 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**, Fig. 1) was obtained as a light-brown amorphous solid. Its molecular formula was established as $C_{34}H_{24}O_{12}$ on the basis of its molecular ion peak in its negative ion 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**, Fig. 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

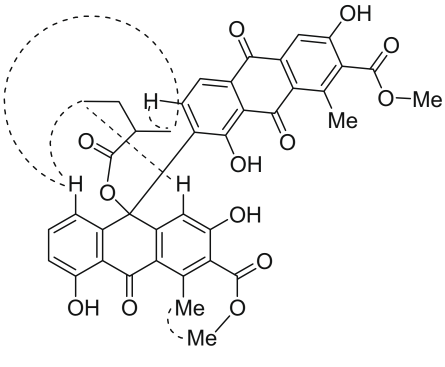
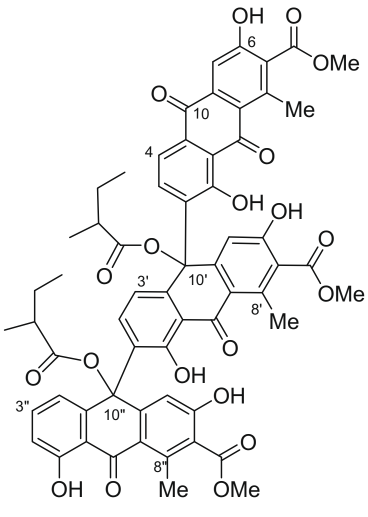


Figure 3. Key ROESY correlations of **1**.



4

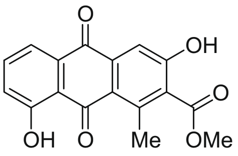
Figure 4. Structure of compound **4**.

mass spectrum. The UV–vis spectra showed characteristic features of anthraquinones at 248, 272, 310, 353 and 475 nm. In the ^{1}H 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 8.33/8.32, d, J = 8.5 Hz, H-3′ and δ_H 6.92, d, J = 8.5 Hz, H-4′) and one ABC 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 ^{1}H 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 ^{1}H 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 ^{1}H 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**, Fig. 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 *P. 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**–**3**, and the monomer **5** showed the weakest activity. Compounds **1**–**5** showed moderate antiplasmodial 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 µM). The selectivities of compounds **1**–**5** for antiplasmodial as opposed to antiproliferative activities, as determined by the quotient of antiproliferative activity and antiplasmodial activity, vary between 0.8 and 6 (Table 3).



5

Figure 5. Structure of compound **5**.

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

3.1. General

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 hertz. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative column (250 × 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 *P. falciparum* FCM29 were performed at the Centre National d'Application des Recherches Pharmaceutiques. Assays with the chloroquine-resistant *P. falciparum* Dd2 were performed at Georgetown University. Both assays used the previously reported SYBR green 1 method.[23]

Table 3
IC$_{50}$ (µM) values of **1**–**5** against Human A2780[a] and *Plasmodium falciparum* strains FCM29 and Dd2

Compound	A2780 IC$_{50}$ (µM)	*P. falciparum* Dd2 IC$_{50}$ (µM)	Selectivity index (Dd2 vs A2780)	*P. falciparum* 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

[a] Paclitaxel (IC$_{50}$ 0.014 µM) was the positive control.[21]

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 IC_{50} value of 12 µg/mL against A2780 cells. This extract (2.0 g) was suspended in aqueous MeOH (90% MeOH–H_2O, 60 mL) and extracted with hexanes (3 × 60 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 90 mL). The CH_2Cl_2 extract (82.2 mg) was found to be the most active against A2780 cells (IC_{50} = 6 µg/mL) and was separated via semipreparative HPLC over a C18 column using MeOH–H_2O (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, $CHCl_3$); UV (MeOH) λ_{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 $C_{39}H_{32}O_{13}$: 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; ^{13}C NMR (CD_3OD): 192.2 (C-9/C-9′), 190.9 (C-9/C-9′), 183.6 (C-10), 174.9 (C-10′-OCOCH(CH_3)CH_2CH_3), 170.6 (C-7-$COOCH_3$), 169.9 (C-7′-$COOCH_3$), 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-$COOCH_3$), 53.0 (C-7′-$COOCH_3$), 43.0 (C-10′-OCOCH(CH_3)CH_2CH_3), 28.0/27.9 (C-10′-OCOCH(CH_3)CH_2CH_3), 21.2 (8′-CH_3), 20.6 (8-CH_3), 17.0/16.9 (C-10′-OCOCH(CH_3)CH_2CH_3), 12.0 (C-10′-OCOCH(CH_3)CH_2CH_3).

3.7. Scutianthraquinone B (2)

Light-brown amorphous solid; $[\alpha]_D^{25}$ +134.8 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{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 $C_{38}H_{30}O_{13}$: 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) λ_{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 $C_{34}H_{24}O_{12}$: 624.1268); LC–ESIMS *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, $CHCl_3$); UV (MeOH) λ_{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 $C_{61}H_{52}O_{20}$: 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) λ_{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 $C_{17}H_{12}O_6$: 312.0634); ^1H NMR (400 MHz, $CDCl_3$) 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-COOCH_3), 2.98 (3H, s, 8-CH_3); ^1H NMR (500 MHz, CD_3OD) 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-COOCH_3), 2.69 (3H, s, 8-CH_3); ^{13}C NMR (500 MHz, CD_3OD) δ 191.1 (C-9), 184.0 (C-10), 169.9 (7-$COOCH_3$), 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-COOCH_3), 20.6 (8-CH_3).

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Supplementary data

Proposed mass spectrometric fragmentation schemes for compounds **1**–**4**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.022.

References and notes

1. Biodiversity Conservation and Drug Discovery in Madagascar, Part 35. For Part 34, see: Hou, Y.; Cao, S.; Brodie, P. J.; Callmander, M.; Ratovoson, F.; Randrianaivo, R.; Rakotobe, E.; Rasamison, V. E.; Rakotonandrasana, S.; TenDyke, K.; Suh, E. M.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2009**, *17*, 2215.
2. Ayers, S.; Zink, D. L.; Mohn, K.; Powell, J. S.; Brown, C. M.; Murphy, T.; Brand, R.; Pretorius, S.; Stevenson, D.; Thompson, D.; Singh, S. B. *J. Nat. Prod.* **2007**, *70*, 425.
3. Morel, A. F.; Maldaner, G.; Ilha, V.; Missau, F.; Silva, U. F.; Dalcol, I. I. *Phytochemistry* **2005**, *66*, 2571.
4. Morel, A. F.; Machado, E. C. S.; Moreira, J. J.; Menezes, A. S.; Mostardeiro, M. A.; Zanatta, N.; Wessjohann, L. A. *Phytochemistry* **1998**, *47*, 125.
5. Morel, A. F.; Van Fossen Bravo, R.; Reis, F. A. M.; Ruveda, E. A. *Phytochemistry* **1979**, *18*, 473.
6. Tschesche, R.; Hillebrand, D. *Phytochemistry* **1977**, *16*, 1817.
7. Mammo, W.; Dagne, E.; Steglich, W. *Phytochemistry* **1992**, *31*, 3577.
8. Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon: Oxford, 1964.
9. Yagi, A.; Makino, K.; Nishioka, I. *Chem. Pharm. Bull.* **1974**, *22*, 1159.
10. Qhotsokoane-Lusunzi, M. A.; Karuso, P. *J. Nat. Prod.* **2001**, *64*, 1368.
11. Qhotsokoane-Lusunzi, M. A.; Karuso, P. *Aust. J. Chem.* **2001**, *54*, 427.
12. Alemayehu, G.; Hailu, A.; Abegza, B. M. *Phytochemistry* **1996**, *42*, 1423.
13. Alemayehu, G.; Abegza, B.; Snatzke, G.; Duddeck, H. *Phytochemistry* **1993**, *32*, 1273.
14. Pepalla, S. B.; Jammula, S. R.; Jagannadha Rao, K. V.; Thomson, R. H. *Phytochemistry* **1992**, *31*, 2103.
15. Conner, J. M.; Gray, A. I.; Waterman, P. G. *J. Nat. Prod.* **1990**, *53*, 1362.
16. Dagne, E.; Berhanu, E.; Steglich, W. *Bull. Chem. Soc. Ethiop.* **1987**, *1*, 32.
17. Wube, A. A.; Bucar, F.; Asres, K.; Gibbons, S.; Rattray, L.; Croft, S. L. *Phytother. Res.* **2005**, *19*, 472.
18. Mutanyatta, J.; Bezabih, M.; Abegaz, B. M.; Dreyer, M.; Brun, R.; Kocherd, N.; Bringmann, G. *Tetrahedron* **2005**, *61*, 8475.
19. Abegaz, B. M.; Bezabih, M.; Msuta, T.; Brun, R.; Menche, D.; Muhlbacher, J.; Bringmann, G. *J. Nat. Prod.* **2002**, *65*, 1117.
20. Bringmann, G.; Mutanyatta-Comar, J.; Maksimenka, K.; Wanjohi, J. M.; Heydenreich, M.; Brun, R.; Muller, W. E. G.; Peter, M. G.; Midiwo, J. O.; Yenesew, A. *Chem. Eur. J.* **2008**, *14*, 1420.
21. Cao, S.; Brodie, P. J.; Randrianaivo, R.; Ratovoson, F.; Callmander, M.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2007**, *70*, 679.
22. Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. *Cancer Res.* **1985**, *45*, 2110.
23. Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D. *Antimicrob. Agents Chemother.* **2004**, *48*, 1807.