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Cardenolides of *Leptadenia madagascariensis* from the Madagascar dry forest ☆

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ABSTRACT

Investigation of the endemic Madagascar plant *Leptadenia madagascariensis* Decne. (Apocynaceae) for antiproliferative activity against the A2780 ovarian cancer cell line led to the isolation of the four new cardenolides **1**–**4**. The structure elucidations of these compounds were based on analyzes of their 1D and 2D NMR spectra and mass spectrometric data. The cardenolides were strongly antiproliferative to the A2780 ovarian cancer cell line, with IC_{50} values of 0.18, 0.21, 0.17, and 0.29 μM line, and to the H460 human lung cancer cell line, with IC_{50} values of 0.16, 0.68, 0.37, and 0.48 μM, respectively.

1. Introduction

In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2,3] we obtained an EtOH extract from the roots of the plant *Leptadenia madagascariensis* Decne. (Apocynaceae) from Madagascar. The extract exhibited good antiproliferative activity against the A2780 human ovarian cancer cell line, with an IC_{50} value of 10 μg/mL. On the basis of this activity and the absence of any previous phytochemical studies on this species, the extract was selected for fractionation to isolate its active components by bioassay-guided fractionation.

There are about 20 species in the genus *Leptadenia*, some of which are used in traditional medicine in Africa and India.[4–6] Previous phytochemical investigations reported the presence of flavonoids,[5,7,8] terpenoids,[1–10] polyoxypregnane esters,[4,11] pregnane glycosides,[12,13] cardiac glycosides,[14] and alkaloids[15] in these species. The medicinal use of plants containing cardiac glycosides was recorded as early as 1500 years ago. As an important class of natural products, cardiac glycosides are widely used for treating cardiac failure,[16] and their cardiac activities and cytotoxicities are well known.[6,17,18] What is less well known is the fact that they are also beginning to find use in cancer chemotherapy, and the first generation of anticancer cardiac glycosides is in clinical trials.[19,20]

2. Results and discussion

The EtOH extract of the stems and leaves of *L. madagascariensis* was subjected to liquid–liquid partitioning to give active dichloromethane and MeOH fractions with IC_{50} values in the A2780 assay of 0.28 and 2.4 μg/mL, respectively. Fractionation by C18 open column and High Performance Liquid Chromatography (HPLC) on the MeOH fraction yielded two new cardenolides named madagascarensilide A (**1**) and madagascarensilide B (**2**) (Fig. 1). Similar purification of the CH_2Cl_2 fraction yielded the two additional new compounds madagascarensilides C and D (**3** and **4**, Fig. 3). Herein we report the structural elucidation and the antiproliferative properties of the four isolates.

Madagascarensilide A (**1**) was obtained as a white amorphous solid. Its positive ion HRESIMS revealed a pseudomolecular ion peak at *m*/*z* 849.4255 $[M+Na]^+$, corresponding to a molecular formula of $C_{42}H_{66}O_{16}$ for **1**. Its ^1H NMR spectrum in CD_3OD showed signals at δ_H 5.04 dd (J = 18.6, 1.8 Hz), 4.92 dd (J = 18.6,1.8 Hz), and 5.90 s, characteristic of an α,β-unsaturated γ-lactone (Table 1). In addition, three anomeric proton signals were observed at δ_H 4.91 dd (J = 9.5, 1.8 Hz), 4.57 d (J = 7.7), and 4.37 d (J = 7.7). The ^{13}C NMR spectrum contained 42 signals, which included signals for 1 methoxyl, 4 methyls, 12 methylenes (including 1 oxymethylene), 20 methines (including 15 oxymethines and 1

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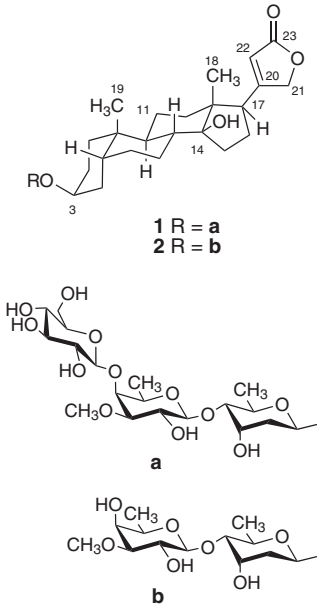

Figure 1. Chemical structures of madagascarensilides A (**1**) and B (**2**).

olefinic carbon), and 5 quaternary carbons (including 1 oxyquaternary carbon, 1 olefinic carbon and 1 carbonyl carbon), as indicated by an HMQC spectrum (Table 1). The above data suggested that **1** is a cardiac glycoside with three sugar moieties.

In the aglycone of **1**, two spin systems CH_2–CH_2–CH–CH_2–CH–CH_2–CH_2–CH–CH–CH_2–CH_2 (H_2-1 through H_2-2, H-3, H_2-4, H-5, H_2-6, H_2-7, H-8, H-9, and H_2-11 to H_2-12) in rings A, B, and C, and CH_2–CH_2–CH (H_2-15 through H_2-16 to H-17) in ring D (Fig. 2) were identified in the COSY and TOCSY spectra. The connectivities of rings A, B, C, and D were assigned based on the interpretation of the HMBC spectrum. Long-range correlations from H_3-19 to C-1, C-5, C-9, and C-10, and from H_2-1 to C-9 indicated the connectivity of rings A and B. The relationship between rings C and D was established by the observation of correlations from H_3-18 to C-12, C-13, the oxygenated quaternary carbon at C-14 and C-17, as well as those observed from H_2-12 to C-17, and H_2-15 to C-8. Moreover, the α,β-unsaturated γ-lactone was deduced to be connected to C-17 by the HMBC correlation from H-17 to C-20, C21, and C22. The Rotating frame Overhauser Effect SpectroscopY (ROESY) correlation between H_3-19 and H-5 indicated that rings A and B are *cis* fused, while the *trans* orientation of H-8 and H-9 was deduced from the presence of correlations between H_3-18 and H_3-19 to H-8 (Fig. 2). The correlations of H_3-18 to C-14-OH, H-21, and H-22 in the ROESY spectrum in deuterated pyridine indicated *cis* fused C and D rings and the β-orientation of the γ-lactone at C-17. These data, together with a comparison of the ^{13}C NMR data of **1** with those of the aglycone of coroloside and similar digitoxigenin glycosides, established the aglycone of **1** as digitoxigenin.[21]

The presence of three sugar units in **1** was indicated by the presence of three anomeric proton signals at δ_H 4.91, 4.57, and 4.37. Their spin systems were determined by COSY and TOCSY correlations: H-1′-H_2-2′-H-3′-H-4′-H-5′-H_3-6′, H-1-H-2″-H-3″-H-4″-H-5″-H_3-6″, and H-1‴-H-2‴-H-3‴-H-4‴-H-5‴-H_2-6‴. In addition, HMBC correlations from H-1′ to C-3, H-1″ to C-4′, and H-1‴ to C-4″ built up the connectivity of the sugar units from C-1′ to C-3, C-1″ to C-4′, and C-1‴ to C-4″. The relative conformations of the sugar moieties were determined by the coupling constants of the sugar protons and by analysis of the ROESY data. In the ^{1}H NMR spectrum of **1** in deuterated pyridine, the coupling constants observed for H-1′ (d, J = 9.5, 1.8) and for H-4′ (dd, J = 9.7, 2.7) as well as the clear ROESY correlation between H-1′ and H-5′ indicated that H-1′, H-4′, and H-5′ are all axial, and so H-3′ must be equatorial based on its 2.7 Hz coupling constant with H-4′. The HMBC correlation between the methoxy protons at 3.69 ppm and C-3″ (δ_C = 85.6) placed the methoxy group at C-3″. In the same manner, the coupling constants observed for H-1″ (d, J = 7.7), H-3″ (dd, J = 9.7, 2.9) and the ROESY correlation of H-5″ with H-1″ indicated that H-1″, H-2″, H-3″, H-5″ are axial, while H-4″ is equatorial. The coupling constants observed for the third sugar unit for H-1‴ (d, J = 7.8) and H-2‴ (t, J = 7.8), with ROESY correlations from H-1‴ to H-3‴ and H-5‴, as well as from H-2‴ to H-4‴, led to the conclusion that all the protons in this sugar unit must be axial, indicating it to be glucopyranose. Therefore, the structure of **1** was determined to be digitoxigenin 3-*O*-β-glucopyranosyl-(1 → 4)-*O*-β-digitalopyranosyl-(1→4)-*O*-β-digitoxopyranoside. The absolute stereochemistry of the glucose unit was assigned as D since L-glucose has never been observed in cardenolides. The digitoxose and digitalose units were also assigned as D-sugars based on their occurrence in other cardenolides in the D-form.[21,22]

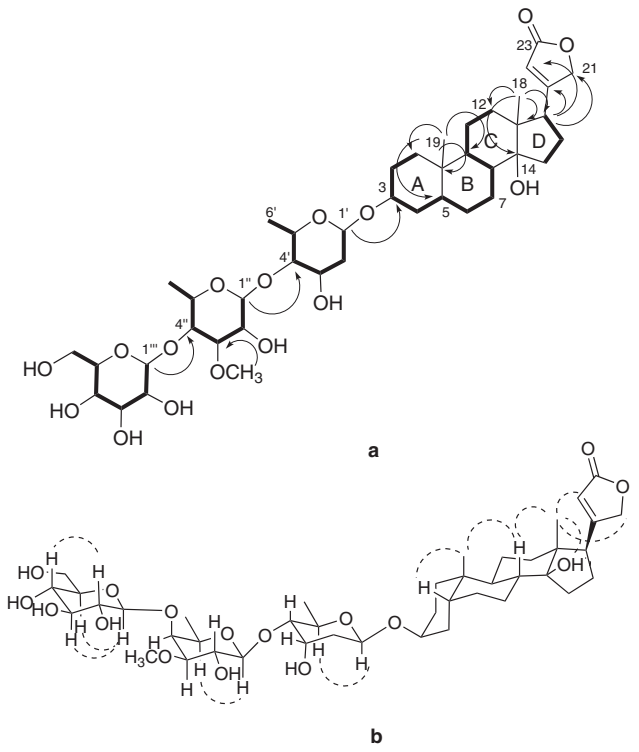

Figure 2. (a) Key COSY (bold) and HMBC (arrows) correlations for **1**. (b) Key ROESY correlations for **1**.

Madagascarensilide B (**2**) was obtained as a white amorphous solid. Its positive ion HRESIMS revealed a pseudomolecular ion peak at m/z 687.3728, corresponding to a molecular formula of $C_{36}H_{56}O_{11}$ for **2**. The ^{1}H NMR data of **2** in CD_3OD were very similar to those of **1**, and thus its structure was indicated to be a cardiac glycoside with two sugar units. The ^{1}H and ^{13}C NMR data arising from the aglycone and the sugar moiety attached at C-3 of **2** were essentially superposable with those of **1**. In addition, the spin system H-1″-H-2″-H-3″-H-4″-H-5″-H_3-6″ was identified by analysis of the COSY data (Table 1). The HMBC correlation observed between the methoxy protons and C-3″ indicated that the second sugar moiety shares the same planar structure as the second sugar of **1**. The coupling constants observed at H-1″ (d, J = 7.7), H-2″ (dd, J = 9.7, 7.7), H-3″ (dd, J = 9.7, 3.1), and H-5″ (br q, J = 6.4), together with the ROESY correlation between H-1″ and H-5″ indicated that H-1″, H-2″, H-3″, and H-5″ are axial while H-4″ is equatorial. Thus

the structure of **2** was assigned as digitoxigenin 3-*O*-β-digitalopyranosyl-(1 → 4)-*O*-β-digitoxopyranoside.

Madagascarensilide C (**3**) was obtained as a white amorphous solid. Its positive ion HRESIMS revealed a pseudomolecular ion peak at *m*/*z* 845.4315 $[M+Na]^+$ corresponding to a molecular formula of $C_{43}H_{66}O_{15}$ for **3**. The ^1H and ^{13}C NMR spectra in deuterated pyridine indicated compound **3** to be a cardenolide with three sugar units, with signals for three anomeric protons at δ_H 5.39, 5.17, and 4.69 ppm and one aldehyde at δ_H 10.42 (Table 1). In the aglycone of **3**, three spin systems: CH_2–CH_2–CH–CH_2 (H_2-1 through H_2-2, H-3 to H_2-4) for ring A; CH_2–CH_2–CH–CH–CH_2–CH_2 (H_2-6 through H_2-7, H-8, H-9, H_2-11 to H_2-12) in rings B and C; and CH_2–CH_2–CH (H_2-15 through H_2-16 to H-17) in ring D were identified by COSY and TOCSY spectra. The connectivities of rings A, B, C and D were assigned based on an analysis of HMBC data. The long-range correlations arising from H-19 at δ_H 10.42 to C-1 and H-9 at δ_H 1.78 to C-19 at δ_C 208.9, as well as the correlations from H_a-4 and H_a-6 to C-10, and from the hydroxyl group signal at C-5 to C-4 and C-6, indicate the connectivity of rings A and B. Meanwhile, the relationship between rings C and D was established by the observation of correlations from H_3-18 to C-12, C-13, C-14, and C-17, and from the hydroxy group at C-14 to C-8, C-13, C-14, and C-15. The α,β-unsaturated γ-lactone was determined to be at C-17 by the HMBC correlation from H-17 to C-20, C21, and C22. Moreover the assigned ^{13}C NMR chemical shifts of the aglycone of **3** in CD_3OD (Table 1) are very similar to those of strophanthidin.[23] From the above data, the planar structure of the aglycone of **3** was deduced to be strophanthidin.

The spin systems H-1′-H_2-2′-H-3′-H-4′-H-5′-H_3-6′, H-1″-H_2-2″-H-3″-H-4″-H-5″-H_3-6″, and H-1‴-H_2-2‴-H-3‴-H-4‴-H-5‴-H_3-6‴ of the sugar units were assigned by COSY and TOCSY correlations. Long-range correlations from H-1′ to C-3, H-1″ to C-4′, and H-1‴ to C-4″ established the connectivity of the sugar units as depicted in Figure 3. The methoxy groups at C-3″ and C-3‴ were substantiated by observation of HMBC correlations in deuterated pyridine between the methoxy signals (δ_H 3.51 and 3.40, each singlet) and the two carbon signals at δ_C 78.1 and 79.3 (C-3″ and C-3‴, respectively). The relative conformations of the sugar moieties were determined by analysis of the ROESY data of **3** and the coupling constants of the sugar protons (Fig. 4). The values of the coupling constants of H-1′ (*J* = 9.6, 1.9 Hz) and H-4′ (*J* = 9.7, 3.0 Hz) and the clear ROESY correlation between H-1′ and H-5′ indicated that H-1′, H-4′, and H-5′ are axial, while H-3′ is equatorial. The coupling constants of H-1″ (dd, *J* = 9.7, 1.8) and H-4″ (dd, *J* = 9.7, 2.7) and the ROESY correlation between H-5″ and H-1″ suggested that H-1″, H-4″, and H-5″ are axial, while H-3″ is equatorial. Similarly, the coupling constants of H-1‴ (dd, *J* = 9.7, 2.1), H-3‴ (ddd, *J* = 12.1, 4.7, 2.9), and H-5‴ (qd, *J* = 6.4, 1.3), and the ROESY correlation between H-1‴ and H-5‴ indicate that H-1‴, H-3‴, and H-5‴ are axial and H-4‴ is equatorial. Therefore, the structure of **3** was determined as strophanthidin 3-*O*-β-diginopyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside.

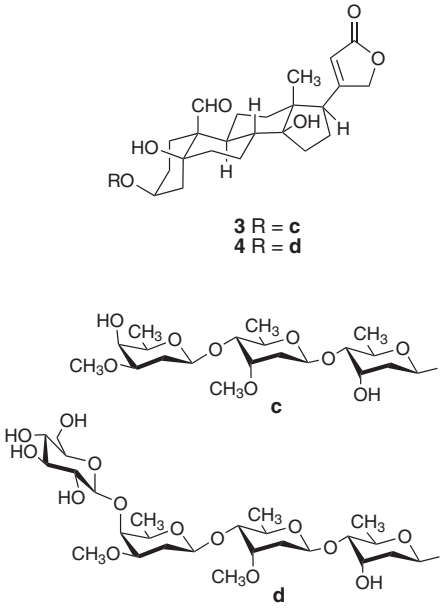

Figure 3. Chemical structures of madagascarensilides C (**3**) and D (**4**).

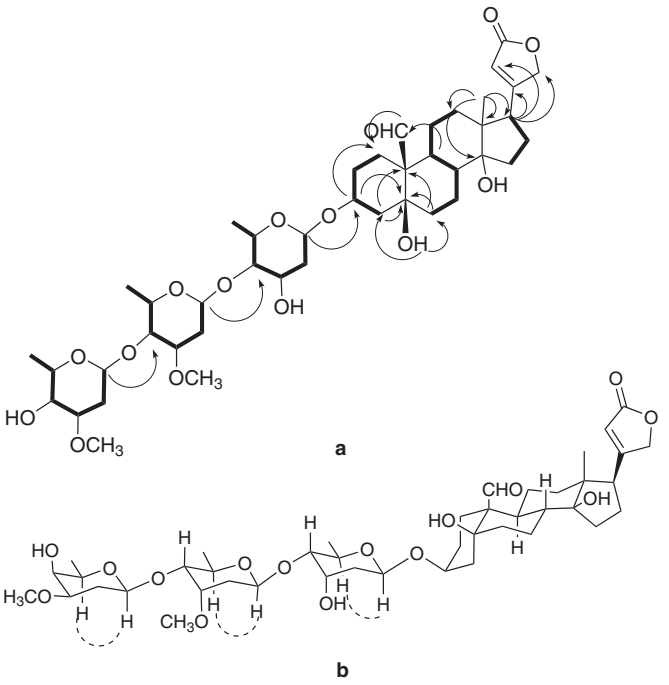

Figure 4. (a) Key COSY (bold) and HMBC (arrows) correlations for **3**. (b) Key ROESY correlations for **3**.

Madagascarensilide D (**4**) was obtained as a white amorphous solid. Its positive ion HRESIMS revealed a pseudomolecular ion peak at *m*/*z* 1007.4844 $[M+Na]^+$ corresponding to a molecular formula of $C_{49}H_{76}O_{20}$ for **4**. The ^1H and ^{13}C NMR spectroscopic data of **4** were very similar to those of **3**, suggesting that **4** is a cardenolide derivative with four sugar units (Table 1). Inspection of the carbon chemical shifts of **4** revealed a close similarity to those of **3** except for the downfield shifts of the signals arising from C-3‴ (+1.4) and C-4‴ (+6.9) and the upfield shift of the signal of C-5‴ (−0.2). These data indicated that the additional sugar unit in **4** is linked to the third sugar, and the position of attachment of this fourth sugar unit was confirmed to be at C-4‴ due to observation of a clear HMBC correlation between H-1⁗ and C-4‴. The ^{13}C NMR data of the fourth sugar unit (δ_C 104.6, 76.0, 78.2, 71.8, 78.0, 63.0) were very similar to the those of the terminal β-glucopyranosyl unit of the tetrasaccharide adoligose B (β-Glc-β-Dgn-β-Cym-α-Cym),[24] indicating the sugar moieties of **4** to be β-Glc-β-Dgn-β-Cym-β-Dgx. Compound **4** is thus strophanthidin 3-*O*-β-glucopyranosyl-(l → 4)-*O*-β-diginopyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside.

3. Bioassay data

Madagascarensilide A (**1**), B (**2**), C (**3**), and D (**4**) were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. Compounds **1** and **3** were the most potent, having an IC_{50} value of 0.18 and 0.17 μM, while compounds **2** and **4** were

slightly less potent, with IC_{50} values of 0.21 and 0.29 μM, respectively. It appears that the aldehyde group at position 10 between rings A and B on the aglycone does not significantly affect the activity of cardenolides against A2780 cells.

Cardenolides **1**–**4** were also evaluated in the H460 human lung cancer cell line. Madagascarensilide A (**1**) showed strong activity with a IC_{50} value of 0.16 μM. Madagascarensilide B (**2**), C (**3**), and D (**4**) were also active with IC_{50} values of 0.68, 0.37, and 0.48 μM, respectively.

4. Experimental

4.1. General experimental procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV and IR spectra were measured on a Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively. NMR spectra were obtained in CD_3OD or deuterated pyridine on either JEOL Eclipse 500 or Bruker Avance 600 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz. Mass spectra were obtained on an Agilent 6220 TOF Mass Spectrometer. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C18 Varian Dynamax column (5 μm, 250 × 10 mm).

4.2. Antiproliferative bioassays

4.2.1. A2780 ovarian cancer cell line

A2780 human ovarian cancer cell[25] were grown to 95% confluency and harvested and resuspended in growth medium (RPMI-1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine). Cells were counted using a hemacytometer and a solution containing 2.5×10^5 cells/mL was prepared in growth media. Eleven columns of a 96 well microtiter plate were seeded with 199 μL of cell suspension per well, and the remaining column contained media only (100% inhibition control). The plate was incubated for 3 h at 37 °C/5% CO_2 to allow the cells to adhere to the wells. Following this incubation, potential cytotoxic agents, prepared in DMSO, were added to the wells in an appropriate series of concentrations, 1 μL per well. One column of wells was left with no inhibitor (0% inhibition control), and four dilutions of a known compound (taxol or actinomycin) was included as a positive control. The plate was incubated for 2 days at 37 °C/5% CO_2, then the media gently shaken from the wells and replaced with reaction media (supplemented growth medium containing 1% alamarBlue), and incubated for another 3 h. The level of alamarBlue converted to a fluorescent compound by living cells was then analyzed using a Cytofluor Series 4000 plate reader (Perseptive Biosystems) with an excitation wavelength of 530 nm, an emission wavelength of 590 nm, and gain of 45. The percent inhibition of cell growth was calculated using the 0% and 100% controls present on the plate, and an IC_{50} value (concentration of cytotoxic agent which produces 50% inhibition) was calculated using a linear extrapolation of the data which lie either side of the 50% inhibition level. Samples were analyzed in triplicate on at least two separate occasions to produce a reliable IC_{50} value.

4.2.2. H460 NSCLC cell line

The cell growth inhibition assay was performed using the H460 (NSCLC) cell line at Eisai Inc. Andover, MA. The cells were cultured in 96-well plates in the absence or continuous presence of test samples at various concentrations for 96 h. Cell growth was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Luminescence was read on the EnVision 2102 Multilabel Reader (Perkin–Elmer). IC_{50} values were determined as the concentration of test sample at which cell growth was inhibited by 50% compared to untreated cell population; vinblastine was used as a reference compound.

4.3. Plant material

A sample of the roots of *L. madagascariensis* Decne. (Apocynaceae) was collected in 2007 2 km west of the village of Ambolobozobe, Madagascar, in degraded dry forest. Collection co-ordinates were 12°31′26″ S, 49°31′29″ E, and elevation 20 m. Voucher specimens have been deposited at the Parc Botanique and Zoologique de Tsimbazaza (TAN) and at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Muséum National d'Histoire Naturelle in Paris, France (P), voucher number SR1092.

4.4. Extraction and isolation

Dried roots of *L. madagascariensis* (275 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at room temperature to give the crude extract MG 4294 (12.7 g), of which 3.34 g was shipped to Virginia Polytechnic Institute and State University (VPISU) for bioassay-guided isolation. The extract MG 4294 (IC_{50} 3.6 μg/mL, 2.0 g) was suspended in aqueous MeOH (MeOH–H_2O, 9:1, 100 mL) and extracted with hexane (3 × 100 mL portions). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 150 mL portions). The hexane extract was evaporated in vacuo to leave 352 mg with an IC_{50} value of 15 μg/mL. The residue from the CH_2Cl_2 extract (223 mg) had an IC_{50} of 0.28 μg/mL and the aqueous MeOH extract (1.22 g) had an IC_{50} of 2.4 μg/mL. Fractionation of the aqueous MeOH extract by C18 open column gave the four fractions I–IV (987.6, 70.4, 11.9, and 5.8 mg), with IC_{50} values of 10, 0.14, 16, and 14 μg/mL, respectively. The most active fraction (fr-II) was separated further by C-18 HPLC (solvent system: MeOH–H_2O 70:30), and compounds **1** (2.8 mg, t_R = 15.5 min) and **2** (0.7 mg, t_R = 21.4 min) were isolated. Fractionation of the CH_2Cl_2 extract on a C18 open column gave the five fractions A–E (11.5, 67.3, 47.8, 44.6, and 8.8 mg) with IC_{50} values: >20, 0.11, 0.12, 0.97, and 6.9 μg/mL, respectively. Fraction B was selected for further purification by C18-HPLC (solvent system: gradient from MeOH: H_2O 60:40 to 70:30 for 40 min) to afford compounds **3** (1.5 mg, t_R 38.7 min) and **4** (5.0 mg, t_R = 28.0 min).

4.5. Digitoxigenin 3-*O*-β-glucopyranosyl-(1 → 4)-*O*-β-digitalopyranosyl(1 → 4)-*O*-β-digitoxopyranoside (1, madagascarensilide A)

Compound **1** was a white amorphous solid; $[\alpha]_D^{23}$ +11 (*c* 0.28, MeOH); UV (MeOH) λ_{max} nm (ε) 215 (4.1); IR ν_{max} cm^{-1}: 3396, 2934, 1739, 1449, 1372, 1073 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, d-pyridine) and ^{13}C NMR (125 MHz, CD_3OD, d-pyridine), see Table 1; HRESI-MS *m*/*z* 849.4255 $[M+Na]^+$ (calcd for $C_{42}H_{66}NaO_{16}$ 849.4249).

4.6. Digitoxigenin 3-*O*-β-digitalopyranosyl-(1 → 4)-*O*-β-digitoxopyranoside (2, madagascarensilide B)

Compound **2** was a white amorphous solid; $[\alpha]_D^{23}$ +16 (*c* 0.07, MeOH); UV (MeOH) λ_{max} nm (ε) 215 (3.6); IR ν_{max} cm^{-1}: 3224, 2940, 1739, 1595, 1355, 1078 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRESI-MS *m*/*z* 687.3728 $[M+Na]^+$ (calcd for $C_{36}H_{56}NaO_{11}$ 687.3720).

4.7. Strophanthidin 3-*O*-β-diginopyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside (3, madagascarensilide C)

Compound **3** was a white amorphous solid; $[\alpha]_D^{23}$ +21 (*c* 0.15, MeOH); UV (MeOH) λ_{max} nm (ε) 215 (3.9); IR ν_{max} cm^{-1}: 3459, 2939, 1740, 1374, 1072 cm^{-1}. ^1H NMR (500 MHz, d-pyridine) and

Table 1
^{1}H and ^{13}C NMR chemical shifts of madagascarensilides A (**1**), B (**2**), C (**3**), and D (**4**)[a]

Position	**1**[b]		**1**[c]		**2**[b]		**3**[c]		**3**[b]		**4**[b]	
	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1} (*J*, Hz)	^{13}C	^{1} (*J*, Hz)	^{13}C	^{1} (*J*, Hz)	^{13}C	^{1} (*J*, Hz)	^{13}C
Aglycone												
1	1.46 m	31.0		30.9		31.0	2.59 m, 1.91 m	18.9	1.72 m, 1.60 m	18.9		18.9
2	1.63 m	27.5		27.5		28.1	2.19 m, 1.68 m	26.0	1.94 m, 1.61 m	25.9		25.9
3	4.02 m	74.5	4.27 m	73.6	4.26 m	74.5	4.33 m	75.3	4.15 m	76.3	4.15 m	76.3
4	1.83 m, 1.46 m	31.4		31.2		31.4	2.19 m, 1.73 m	36.2	2.17 m, 1.62 m	36.8		36.8
5	1.66 m	38.0		37.4		38.0	OH 4.89s	74.1		75.2		75.2
6	1.88 m, 1.26 m	27.9		27.5		27.5	2.34 m, 1.82 m	37.3	2.17 m, 1.62 m	37.2		37.2
7	1.78 m, 1.25 m	22.4		22.0		22.4	2.33 m, 1.46 m	25.2	2.12 m, 1.32 m	25.2		25.2
8	1.63 m	42.7		42.3		42.7	2.31 m	42.3	1.94 m	42.6		42.6
9	1.73 m	36.9		36.3		36.9	1.78 m	39.9	1.66 m	40.4		40.4
10		36.3		35.9		36.3		56.1		56.1		56.1
11	1.43 m, 1.24 m	22.6		22.4		22.6	1.59 m, 1.40 m	23.0	1.56 m, 1.51 m	23.3		23.3
12	1.51 m	41.0		40.3		41.0	1.45 m, 1.35 m	39.9	1.49 m, 143 m	40.5		40.5
13		51.1		50.5		51.1		50.2		50.7		50.7
14		86.5		85.0		86.5	–OH, 5.66	84.8		85.9		85.9
15	2.18, 1.73	33.4		33.6		33.4	2.08 m, 1.86 m	32.5	2.17 m, 1.72 m	32.4		32.4
16	2.18, 1.88	28.1		27.7		27.9	2.10 m, 2.02 m	27.6	2.19 m, 2.14 m	27.9		27.9
17	2.83 m	52.1	2.79 m	51.9	2.83 m	52.1	2.79 m	51.5	2.82 m	51.7	2.82 m	51.8
18	0.88 s	16.4	0.89 s	16.6	0.88 s	16.4	1.01	16.4	0.85 s	16.2	0.85 s	16.2
19	0.94 s	24.3	1.02 s	24.3	0.95 s	24.3	10.42	208.9	10.05 s	209.9	10.05 s	209.9
20		178.5		176.4		178.5		176.1		178.2		178.2
21	5.04 dd (18.6, 1.8)	75.4	5.34 dd (18.2, 1.4)	74.1	5.03 dd (18.4, 1.5)	75.4	5.31 dd (18.2, 1.7)	74.4	5.03 dd (18.5, 1.7)	75.3	5.03 dd (18.5, 1.7)	75.3
	4.92 dd (18.6,1.8)		5.06 dd (18.2,1.4)		4.92 dd (18.4,1.5)		5.05 dd (18.2, 1.7)		4.91dd (18.5, 1.7)		4.91dd (18.5, 1.7)	
22	5.90 s	117.8	6.15 s	118.1	5.90 s	117.8	6.14	118.2	5.90	117.9	5.90	117.9
23		177.3		174.9		177.3		174.9		177.2		177.2
Sugar I												
1′	4.91 dd (9.5, 1.8)	96.8	5.44 dd (9.5, 1.8)	96.9	4.91 dd (9.4, 1.5)	96.8	5.39 dd (9.6, 1.9)	98.0	4.91 dd (8.9, 1.9)	98.3	4.91 dd (8.9, 1.9)	98.3
2′	1.95 m, 1.73 m	39.0	2.43 m, 2.11 m	39.9	1.95 m, 1.73 m	38.9	2.34 m, 1.93 m	39.1	1.98 m, 1.71 m	38.7	1.98 m, 1.71 m	38.7
3′	4.24 m	68.7	4.71 m	68.1	4.26 m	68.7	4.61 m	67.8	4.24 m	68.3	4.24 m	68.3
							–OH, 5.52					
4′	3.23 m	84.3	3.67 dd (9.7, 2.7)	84.7	3.23 dd (9.5, 2.9)	84.1	3.50 dd (9.7, 3.0)	83.2	3.23 dd (9.5, 2.9)	83.5	3.23 m	83.5
5′	3.85 m	69.7	4.37 m	69.3	3.85 m	69.7	4.26 m	69.2	3.80 m	69.6	3.80 m	69.6
6′	1.31 d (6.5)	18.6	1.67 d (6.2).	19.2	1.29 d (6.2)	18.5	1.41d (6.3)	18.9	1.21 d (6.2)	18.4	1.21 d (6.1)	18.4
Sugar II												
1″	4.37 d (7.7)	106.2	4.77 d (7.7)	106.7	4.34 (7.7)	106.2	5.17 dd (9.7, 1.8)	100.1	4.84 m	100.7	4.84 m	100.6
2″	3.66 m	71.4	4.42 m	71.7	3.55 dd (9.7, 7.7)	71.4	2.28 m, 1.76 m	36.9	2.15 m, 1.62 m	36.2	2.15 m, 1.62 m	35.8
3″	3.25 m	85.3	3.57 dd (9.7, 2.9)	85.6	3.12 dd (9.7, 3.1)	84.4	4.07 m	78.1	3.87 m	78.5	3.87 m	78.2
4″	4.16 m	76.3	4.28 m	77.8	3.85 m	68.6	3.48 dd (9.7, 2.7)	83.3	3.30 m	83.5	3.30 m	83.7
5″	3.66 m	71.8	3.75 m	71.0	3.63 bq (6.4)	71.6	4.20 m	69.5	3.86 m	70.1	3.86 m	70.0
6″	1.29 d (6.5)	17.4	1.55 d (6.4)	17.9	1.27 d (6.4)	17.0	1.34 d (6.3)	18.9	1.22 d (6.2)	18.6	1.22 d (6.2)	18.6
Sugar III												
1‴	4.57 d (7.7)	103.9	5.10 d (7.8)	106.4			4.69 dd (9.7, 2.1)	103.1	4.55 dd (9.7, 2.0)	103.4	4.57 dd (9.7, 1.9)	103.4
2‴	3.22 m	76.0	4.02 t (7.8)	76.6			2.17 m, 2.29 m	33.1	1.93 m, 1.65 m	32.9	2.00 m, 1.81 m	33.2
3‴	3.37 m	78.2	4.26 m	79.1			3.41 ddd (12.1, 4.7, 2.9)	79.3	3.35 m	79.2	3.47 m	80.6
4‴	3.26 m	71.8	4.25 m	72.2			3.91 m	67.1	3.67 m	67.7	3.99 m	74.6
							–OH 6.00					
5‴	3.26 m	78.0	3.98 m	78.9			3.56 qd (6.4, 1.3)	71.9	3.49 qd (6.6, 1.3)	72.0	3.53 m	71.8
6‴	3.88 m, 3.65 m	63.0	4.59 m, 4.38 m	63.4			1.55 d (6.4)	17.9	1.28 d (6.6)	17.2	1.30 d (6.4)	17.7
Sugar IV												
1⁗											4.56 d (7.7)	104.6
2⁗											3.22 m	76.0
3⁗											3.36 m	78.2
4⁗											3.27 m	71.8

5‴′									3.25 m	78.0
6‴′									3.86 m, 3.64 m	63.0
3′-OCH_3	3.52 s	58.8	3.69 s	59.5	3.46	57.3	3.51 s	59.1	3.44 s	58.4	3.42 s	58.0
3‴-OCH_3							3.40 s	55.7	3.38 s	55.8	3.41 s	56.6

[a] δ (ppm) 500 MHz for ^{1}H and 125 MHz for ^{13}C; multiplicities; *J* values (Hz) in parentheses.
[b] In CD_3OD.
[c] In deuterated pyridine.

^{13}C NMR (125 MHz, d-pyridine), see Table 1; HRESI-MS *m*/*z* 845.4315 $[M+Na]^+$ (calcd for $C_{43}H_{66}NaO_{15}$ 845.4299).

4.8. Strophanthidin 3-*O*-β-glucopyranosyl-(1 → 4)-*O*-β-diginopyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside (4, madagascarensilide D)

Compound **4** was a white amorphous solid; $[\alpha]_D^{23}$ +16 (*c* 0.51, MeOH); UV (MeOH) λ_{max} nm (ε) 216 (4.1); IR ν_{max} cm^{-1}: 3445, 2937, 1736, 1372, 1074 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRESI-MS *m*/*z* 1007.4844 $[M+Na]^+$ (calcd for $C_{49}H_{76}NaO_{20}$ 1007.4828).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.021.

References and notes

1. Pan, E.; Harinantenaina, L.; Brodie, P. J.; Miller, J. S.; Callmander, M. W.; Rakotonandrasana, S.; Rakotobe, E.; Rasamison, V. E.; Kingston, D. G. I., *J. Nat. Prod.*, 2010, doi:10.1021/np100411d.
2. Harinantenaina, L.; Brodie, P. J.; Slebodnick, C.; Callmander, M. W.; Rakotobe, E.; Randrianasolo, S.; Randrianaivo, R.; Rasamison, V. E.; TenDyke, K.; Shen, Y.; Suh, E. M.; Kingston, D. G. I. J. *Nat. Prod.* **2010**, *73*, 1559.
3. Cao, S.; Kingston, D. G. I. *Pharm. Biol.* **2009**, *47*, 809.
4. Aquino, R.; Peluso, G.; DeTommasi, N.; DeSimone, F.; Pizza, C. *J. Nat. Prod.* **1996**, *59*, 555.
5. El-Moghazy, A. M.; Ali, A. A.; El-Sayyad, S. M.; Sayed, H. M. *Fitoterapia* **1980**, *51*, 321.
6. Lhinhatrakool, T.; Sutthivaiyakit, S. *J. Nat. Prod.* **2006**, *69*, 1249.
7. Sankara Subramanian, S.; Nair, A. G. R. *Phytochemistry* **1968**, *7*, 1703.
8. Krishna, P. V. G.; Rao, E. V.; Rao, D. V. *Planta Med.* **1975**, *27*, 395.
9. Nikiema, J. B.; VanhaelenFastre, R.; Vanhaelen, M. *Planta Med.* **1997**, *63*, 486.
10. Nikiema, J. B.; Vanhaelen-Fastre, R.; Vanhaelen, M.; Fontaine, J.; De Graef, C.; Heenen, M. *Phytother. Res.* **2001**, *15*, 131.
11. Aquino, R.; Pizza, C.; Detommasi, N.; Desimone, F. *J. Nat. Prod.* **1995**, *58*, 672.
12. Srivastav, S.; Deepak, D.; Khare, A. *Tetrahedron* **1994**, *50*, 789.
13. Cioffi, G.; Sanogo, R.; Vassallo, A.; Dal Piaz, F.; Autore, G.; Marzocco, S.; De Tommasi, N. *J. Nat. Prod.* **2006**, *69*, 625.
14. Moustafa, A. M. Y.; Khodair, A. I.; Saleh, M. A. *Pharm. Biol.* **2009**, *47*, 826.
15. Moustafa, A. M. Y.; Khodair, A. I.; Saleh, M. A. *Pharm. Biol.* **2009**, *47*, 994.
16. Mehanna, A. S. Cardiac Agents: Cardiac Glycosides, Antianginal, and Antiarrhythmic Drugs. In *Foye's Principles of Medicinal Chemistry*, 6th ed.; Lemke, T. L.; Williams, D. A., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2008; pp 698–721.

17. Rao, R. V.; Vaidyanathan, C. S. *J. Indian Inst. Sci.* **1991**, *71*, 329.
18. Deepak, D.; Srivastava, S.; Khare, N. K.; Khare, A. *Prog. Chem. Org. Nat. Prod.* **1996**, *69*, 71.
19. Newman, R. A.; Yang, P. Y.; Pawlus, A. D.; Block, K. I. *Mol. Interv.* **2008**, *8*, 36.
20. Prassas, I.; Diamandis, E. P. *Nat. Rev. Drug Discovery* **2008**, *7*, 926.
21. Nakamura, T.; Goda, Y.; Sakai, S.; Kondo, K.; Akiyama, H.; Toyoda, M. *Phytochemistry* **1998**, *49*, 2097.
22. Abe, F.; Yamauchi, T. *Chem. Pharm. Bull.* **1979**, *27*(7), 1604.
23. Kopp, B.; Krenn, L.; Kubelka, E.; Kubelka, W. *Phytochemistry* **1992**, *31*, 3195.
24. Pauli, G. F. *J. Nat. Prod.* **1995**, *58*, 483.
25. 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.