Antiproliferative Cassane Diterpenoids of *Cordyla madagascariensis* ssp. *madagascariensis* from the Madagascar Rainforest[1]

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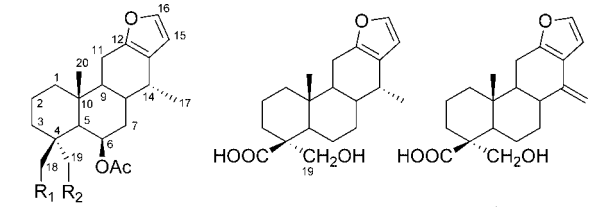
Bioassay-guided fractionation of an ethanol extract of a Madagascar collection of the fruits of *Cordyla madagascariensis* ssp. *madagascariensis* led to the isolation of the four new cassane diterpenoids **1**−**4**. The ^{1}H and ^{13}C NMR spectra of all compounds were fully assigned using a combination of 2D NMR experiments, including COSY, HSQC, HMBC, and ROESY sequences. All of the isolates were tested against the A2780 human ovarian cancer cell line, and compounds **1** and **2** showed mild antiproliferative activity with IC_{50} values of 10 and 36 μM, respectively.

In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] we obtained an extract from the fruit of a plant originally identified as a *Dalbergia* sp. but later reidentified as *Cordyla madagascariensis* ssp. *madagascariensis*; both the *Cordyla* and *Dalbergia* genera belong to the Fabaceae family. The plant was collected in Madagascar, and its extract showed moderate antiproliferative activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 21 μg/mL. No previous work has been reported on the chemistry of *Cordyla* sp. except for the isolation of some flavonoids from the heartwood of *Cordyla africana*,[3,4] and no chemical work has been reported on *Cordyla madagascariensis.* The extract was selected for investigation on the basis of its antiproliferative activity, and bioassay-guided fractionation yielded four new cassane diterpenoids. Herein we report the structural elucidation of these diterpenoids and their bioactivities against the A2780 human ovarian cancer cell line.

Cordylane A (**1**) was obtained as a white powder. Its molecular formula was established as $C_{24}H_{34}O_6$ on the basis of a molecular ion peak at *m/z* 418.2331 in its HRFAB mass spectrum. Its ^{1}H NMR spectrum in C_6D_6 (Table 1) showed signals for one secondary methyl group (δ_H 0.83, d, J = 6.8 Hz, H-17), one tertiary methyl group (δ_H 1.02, s, H-20), and two acetoxy methyl groups (δ_H 1.82, s; 1.64, s). The signals of two oxymethylene groups could also be observed (δ_H 4.72, d, J = 11.4 Hz, H-18a; δ_H 4.27, d, J = 11.4 Hz, H-18b and δ_H 3.47, d, J = 10.8 Hz, H-19a; δ_H 3.24, d, J = 10.8 Hz, H-19b). In addition, the signal of one oxymethine proton was observed in the low-field region of the spectrum (δ_H 5.71, ddd, J = 3.0, 3.0, 2.0 Hz, H-6). A disubstituted furan ring was also present, as evidenced by characteristic resonances (δ_H 7.16, d, J = 2.0 Hz, H-16; δ_H 6.04, d, J = 2.0 Hz, H-15). In the ^{13}C NMR spectrum, the carbons of the furan ring (δ_C 149.6, C-12; 122.6, C-13; 109.7, C-15; 140.8, C-16), a tertiary carbon (δ_C 69.9, C-6), two secondary carbons (δ_C 64.7, C-18; 67.9, C-19), and two ester carbonyl carbons (δ_C 169.8, C-6-OCOCH$_3$; 170.4, C-18-OCOCH$_3$) were identified (Table 2). The complete ^{1}H and ^{13}C NMR assignments and connectivities were determined from a combination of COSY, HSQC, and HMBC data. The COSY spectrum showed correlations that indicated the connectivity of H-5, H-6, H$_2$-7, H-8, H-9, and H$_2$-11, in addition to demonstrating the connectivity of H-8, H-14, and H$_3$-17. These data allowed the assembly of the B and C rings. In the HMBC spectrum, H-18a and H-18b showed correlations to the quaternary C-4 (δ_C 42.5) and to one ester carbonyl carbon at δ_C 170.4, while H-19a and H-19b also showed correlations to C-4. These correlations indicated that the two oxymethylene groups were connected to C-4 and that one *O*-acetyl group was positioned at C-18. The key COSY and HMBC correlations are shown in Figure 1. Furthermore, it was apparent that the other acetyl group was connected to the oxygenated C-6. Analysis of the coupling constants and ROESY correlations enabled determination of the relative configuration at C-4, C-6, C-10, and C-14. The key ROESY correlations are shown in Figure 2. The ROESY correlations from H$_3$-20 to H-2$_{ax}$ (δ_H 1.33, m), H-8 (δ_H 1.84, m), H-11$_{ax}$ (δ_H 2.38, dd, J = 17.6, 10 Hz), and H$_2$-18, from H-5 (δ_H 1.56, d, J = 2.0 Hz) to H-3$_{ax}$ (δ_H 1.16, m) and H-9 (δ_H 1.40, m), from H-9 to H-7$_{ax}$ (δ_H 1.88, m) and H$_3$-17, from H$_2$-19 to H-5 and H-6, and from H-19b to H-3$_{ax}$ indicated that rings A and B had chair conformations with *trans*-fused ring junctions,[5] and thus confirmed the relative configuration at C-4, C-6, C-10, and C-14. The configuration of C-6 was also indicated by the coupling constants of H-6 (J = 3.0, 3.0, 2.0 Hz), which showed that H-6 was in the equatorial position of the chair conformation of ring B, and thus that the *O*-acetyl group at C-6 existed in the β-axial orientation. Therefore, the structure of **1** was assigned as 6β-acetoxycassa-18β-acetoxy-12,15-dien-19α-ol.



1 R_1 = OAc R_2 = OH
2 R_1 = OH R_2 = OAc

3 **4**

Cordylane B (**2**) was obtained as a white powder. Its molecular formula was established as $C_{24}H_{34}O_6$, identical to that of **1**, on the basis of a molecular ion peak at *m/z* 418.2366 in its HRFAB mass spectrum. Nearly identical resonances were observed in the ^{1}H NMR spectra of **2** and **1**, with the exception of the difference in chemical shift of two oxymethylene groups, H$_2$-18 and H$_2$-19. To avoid the overlapping of the ^{1}H NMR signals of H-16 in C_6D_6, further one- and two-dimensional NMR data of **2** were also obtained in CD_3OD, and the following discussion is based on these data. The additional 2D-NMR experiments indicated that H$_2$-19 (δ_H 4.11, d, J = 11.6 Hz and

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Table 1. ^{1}H NMR Data of Compounds **1**−**4**[c]

no.	**1**[a]	**2**[b]	**3**[b]	**4**[b]
1	1.34 m	1.76 m	1.82 m	1.82 m
	0.72 ddd (15.0, 15.0, 5.0)	1.17 m	1.11 m	1.06 m
2	1.49 m	1.69 m	1.97 m	1.97 m
	1.33 m	1.56 m	1.56 m	1.56 m
3	1.67 m	1.93 m	2.77 m	2.26 m
	1.16 m	1.80 m	1.22 m	1.19 m
5	1.56 d (2.0)	1.62 d (2.0)	1.36 dd (12.4, 2.4)	1.37 m
6	5.71 ddd (3.0, 3.0, 2.0)	5.46 ddd (2.8, 2.8, 2.0)	1.88 m	1.90 m
			1.72 m	1.80 m
7	1.88 m	1.86 m	1.71 m	2.34 m
	1.44 m	1.59 m	1.41 m	1.30 m
8	1.84 m	1.97 m	1.75 m	2.20 m
9	1.40 m	1.64 m	1.52 m	1.39 m
11	2.49 dd (17.6, 8.0)	2.62 dd (16.8, 7.2)	2.57 dd (16.4, 6.4)	2.73 dd (16.8, 5.2)
	2.38 dd (17.6, 10.0)	2.44 dd (16.8, 10.0)	2.32 m	2.43 br d (16.8)
14	2.34 m	2.55 m	2.59 m	
15	6.04 d (2.0)	6.18 d (1.6)	6.16 d (1.2)	6.45 d (2.0)
16	7.16 d (2.0)	7.23 d (1.6)	7.21 d (1.2)	7.26 d (2.0)
17	0.83 d (6.8)	0.96 d (7.2)	0.97 d (7.2)	5.07 d (1.6)
				4.88 d (1.6)
18	4.72 d (11.4)	4.07 d (11.2)		
	4.27 d (11.4)	3.69 d (11.2)		
19	3.47 d (10.8)	4.11 d (11.6)	3.83 d (10.6)	3.83 d (10.4)
	3.24 d (10.8)	3.89 d (11.6)	3.46 d (10.6)	3.47 d (10.4)
20	1.02 s	1.20 s	0.88 s	0.93 s
6-OCO*CH$_3$*	1.82 s	2.05 s		
18/19-OCO*CH$_3$*	1.64 s	2.05 s		

[a] In C_6D_6. [b] In CD_3OD. [c] δ (ppm) 400 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**

no.	**1**[a,c]	**2**[a,c]	**2**[b,d]	**3**[b,d]	**4**[b,d]
1	41.1	41.3	42.5	40.8	40.4
2	18.2	18.1	18.8	20.1	20.1
3	30.6	30.4	31.2	33.2	33.1
4	42.5	43.6	44.1	51.0	51.0
5	50.3	54.3	53.7	51.2	50.6
6	69.9	71.6	72.0	24.4	24.1
7	36.4	36.5	37.0	32.7	31.8
8	31.1	31.1	32.3	37.2	37.6
9	46.2	46.1	47.2	40.4	53.3
10	37.8	38.1	39.0	38.7	38.8
11	22.3	22.3	22.8	23.4	23.8
12	149.6	149.5	150.3	150.6	153.5
13	122.6	122.6	123.4	123.5	120.0
14	31.4	31.4	32.4	32.9	144.3
15	109.7	109.7	110.3	110.3	107.2
16	140.8	140.9	141.6	141.5	142.6
17	17.8	17.8	17.9	17.8	104.0
18	64.7	60.8	61.4	179.7	179.6
19	67.9	70.5	71.4	70.9	70.8
20	17.9	17.9	18.6	14.2	14.0
6-O*CO*CH$_3$	169.8	171.2	172.9		
6-OCO*CH$_3$*	21.4	21.3	21.8		
18/19-O*CO*CH$_3$	170.4	169.4	172.0		
18/19-OCO*CH$_3$*	20.4	20.7	20.9		

[a] In C_6D_6. [b] In CD_3OD. [c] δ (ppm) 100 MHz. [d] δ (ppm) 125 MHz.

δ_H 3.89, d, J = 11.6 Hz) had an HMBC correlation to an acetoxy carbonyl group (δ_C 172.0). In addition, H-18a (δ_H 4.07, d, J = 11.2 Hz) showed a ROESY correlation to H_3-20 (δ_H 1.20, s). These data indicated that **2** differed from **1** only in the configuration at C-4. By similar arguments to those used above for **1**, the structure and relative configuration of **2** were determined as 6β-acetoxycassa-19α-acetoxy-12,15-dien-18β-ol.

Cordylane C (**3**) was obtained as a white powder. Its molecular formula was established as $C_{20}H_{28}O_4$ on the basis of a molecular ion peak at *m/z* 332.1988 in its HRFAB mass spectrum. Comparison of the ^{1}H NMR spectra of **1** and **3** showed that the two compounds were very similar, but that the oxymethine (H-6), oxymethylene (H_2-18), and both acetoxy methyl groups that appeared in the ^{1}H NMR spectrum of **1** were absent in the ^{1}H NMR spectrum of **3**. This suggested that

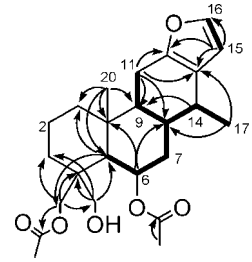

Figure 1. Key COSY (bold) and HMBC (arrows) correlations of **1**.

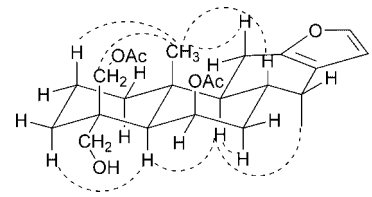

Figure 2. Key ROESY correlations of **1**.

3 had a similar structure to **1** with the exception of the aforementioned functional groups. After further analysis of 2D NMR data, it was found that the oxymethylene protons H_2-19 (δ_H 3.83, d, J = 10.6 Hz and δ_H 3.46, d, J = 10.6 Hz) of **3** showed an HMBC correlation with the carbonyl carbon (δ_C 179.7, C-18) of a carboxylic acid. This indicated that the oxymethylene and carbonyl groups must be connected to the same carbon (C-4). ROESY correlations from H_2-19 to H-6$_{eq}$ (δ_H 1.88, m) and from H-19b to H-3$_{ax}$ (δ_H 1.22, m) and H-5 (δ_H 1.36, dd, J = 12.4, 2.4 Hz) indicated that the C-19 oxymethylene group was in the α-orientation. As in the case of **1**, the full structure and relative configuration of **3** was determined by a combination of one- and two-dimensional NMR and mass spectroscopic methods. Its structure was thus established as cassa-12,15-dien-19α-hydroxy-18β-oic acid.

Cordylane D (**4**) was obtained as a white powder. Its molecular formula was established as $C_{20}H_{26}O_4$ on the basis of its molecular ion peak at *m/z* 330.1841 in its HRFAB mass spectrum. Compound **4** had a similar ^{1}H NMR spectrum to that of **3**, except that the resonance of the methyl group (δ_H 0.97, d, J = 7.2 Hz, H_3-17) in the ^{1}H NMR

spectrum of **3** was replaced by signals for an olefinic methylene group (δ_H 5.07, d, J = 1.6 Hz and δ_H 4.88, d, J = 1.6 Hz, H_2-17). These protons were connected to an olefinic carbon at C-17 (δ_C 104.0) via an HSQC correlation, indicating that the C-17 methyl group in **3** was converted to a vinylic methylene in **4**. This was confirmed by HMBC correlations between both of the methylene hydrogens to an olefinic carbon (δ_C 120.0, C-13), and a methine carbon (δ_C 37.6) assigned as C-8. The structure of **4** was assigned by a combination of one- and two-dimensional NMR and MS methods and by comparison with the data of compound **3**. Its structure was assigned as cassa-12,14(17),15-trien-19α-hydroxy-18β-oic acid.

An interesting phenomena observed in this study was that compound **1** was unstable under acidic conditions. Although compound **1** was stable in CD_3OD and C_6D_6, when it was dissolved in an old sample of $CDCl_3$ for a ^1H NMR experiment, the spectrum showed the presence of a mixture of **1** and **2** even after a short period of time. Presumably the trace amounts of DCl formed on storage of $CDCl_3$ catalyzed the transesterification reaction in **1**. Given this observation, it is not possible to state unambiguously that both **1** and **2** are natural products, since conceivably one could have been converted to the other during extraction in Madagascar or during shipment to the USA and storage.

All the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. It was found that **1** and **2** showed mild antiproliferative activity with IC_{50} values equal to 10 and 36 μM, respectively. Compounds **3** and **4** did not show any significant antiproliferative activity, and both had IC_{50} values greater than 60 μM. It was previously reported that some cassane diterpenoids that had similar structures to the compounds isolated in our work showed moderate cytotoxicity against several cancer cell lines.[6]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were performed on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. Mass spectra were obtained on a JEOL-JMS-HX-110 instrument. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. HPLC was performed using Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative phenyl column (250 × 21.4 mm) and Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative column (250 × 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector.

Antiproliferative Bioassay. The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[7] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[8]

Plant Material. The plant sample of *Cordyla madagascariensis* R. Vig. ssp. *madagascariensis* was collected on March 30, 2004, by R.R. et al. in Antsiranana, Madagascar, in the vicinity of Mahavanoma (12°23′10″ S 49°20′19″ E). It was assigned collector number R. Rakotondrajaona 319, and it was determined by R.R. The specimens were from a tree 15 m high and diameter at chest height of 40 cm. 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).

Extraction and Isolation. Dried fruits from the tree described above (245 g) were extracted with EtOH to give 24.1 g of extract, which was assigned the number MG2180. A total of 1.64 g of extract was supplied to VPISU, and this had an IC_{50} value of 21 μg/mL against A2780 cells. A portion of this extract (1.0 g) was suspended in aqueous MeOH (90% MeOH−H_2O, 40 mL) and extracted with hexanes (3 × 40 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 60 mL). The CH_2Cl_2 extract (401.9 mg) was found to be the most active (IC_{50} = 16 μg/mL) and was separated via preparative HPLC over a Phenyl column using MeOH−H_2O (70:30) to afford 11 fractions (I−XI), of which fractions VI−VIII were found to display the highest antiproliferative activity (IC_{50} = 12, 13, and 12 μg/mL, respectively). Fraction VI was further separated via semi-preparative HPLC on a Phenyl column using MeOH−H_2O (70:30). Six fractions (A−F) were collected. Fraction D afforded cordylane B (**2**, 2.0 mg, t_R 26.7 min), while fraction E afforded cordylane A (**1**, 2.8 mg, t_R 28.6 min). Fraction B was then subjected to semipreparative HPLC on an RP-C18 column using MeOH−H_2O (70:30) to afford six fractions (G−L). Fraction I afforded cordylane D (**4**, 1.4 mg, t_R 25.5 min), and fraction K afforded cordylane C (**3**, 2.0 mg, t_R 28.6 min). Fraction VII was separated using semipreparative RP-C18 HPLC using MeOH−H_2O (75:25). Five fractions (M−Q) were collected. Fraction P afforded cordylane B (**2**, 2.0 mg, t_R 27.3 min). Fraction VIII was also separated using semipreparative phenyl HPLC using MeOH−H_2O (70:30) to obtain five fractions (R−V), of which fraction T afforded cordylane A (**1**, 4.6 mg, t_R 24.9 min).

Cordylane A (1): white powder; $[\alpha]^{23}_D$ +17.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (3.63) nm; IR ν_{max} 3372, 2945, 2831, 1738, 1461, 1370, 1239, 1026 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 418.2331 $[M]^+$ (calcd for $C_{24}H_{34}O_6$, 418.2355).

Cordylane B (2): white powder; $[\alpha]^{23}_D$ +15.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.28) nm; IR ν_{max} 3339, 2945, 2831, 1729, 1461, 1370, 1239, 1029 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 418.2366 $[M]^+$ (calcd for $C_{24}H_{34}O_6$, 418.2355).

Cordylane C (3): white powder; $[\alpha]^{23}_D$ +68.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (3.58) nm; IR ν_{max} 3320, 2951, 2834, 1710, 1468, 1029 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 332.1988 $[M]^+$ (calcd for $C_{20}H_{28}O_4$, 332.1988).

Cordylane D (4): white powder; $[\alpha]^{23}_D$ +40.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.35) nm; IR ν_{max} 3300, 2940, 1710, 1475, 2833, 1030 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m/z* 330.1841 $[M]^+$ (calcd for $C_{20}H_{26}O_4$, 330.1831).

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Supporting Information Available: ^1H NMR spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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