

Antimicrobial Prenylated Anthracene Derivatives from the Leaves of *Harungana madagascariensis*

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Three new prenylated anthranoids, harunmadagascarins C (**1**) and D (**2**) and kenganthranol D (**3**), together with three known compounds (**4**–**6**) were isolated from the leaves of *Harungana madagascariensis*. Their structures were assigned by spectroscopic methods and by comparison with literature data. In the three new natural products **1**–**3**, one or two prenyl groups are incorporated in furan, pyran, or cyclohexane rings in four different modes of annulation. Compounds **2**, **4**, and **6** were strongly active against the Gram-positive *Bacillus megaterium*.

The family Hypericaceae has more than 300 species,¹ and *Harungana madagascariensis* Lam. ex. Poir., called "Nketto" by the Bamileke tribe, represents the only species of this genus in Cameroon.² Interest in the chemical investigation of this plant is mainly due to the biological activities of its extracts and the diversity of its secondary metabolites.^{3–11} The most widely studied constituents are from the class of anthranoids. In our previous studies of the stem bark of this plant, several secondary metabolites were isolated and found to be antioxidants and α -glucosidase inhibition agents.^{12,13} Although several compounds were isolated by other researchers,^{6–11} the constituents of the leaves of the plant are less investigated. As part of our ongoing search for biologically active metabolites from Cameroonian medicinal plants, we now report on the less polar constituents of the leaves of this plant.

Results and Discussion

The hexane extract of the leaves of *H. madagascariensis*, upon column chromatography and further purification of the chromatographic fractions by preparative TLC, afforded three new anthranoids, **1**–**3** (Figure 1).

Anthranoid **1**, named harunmadagascarin C, was obtained as an orange oil, $[\alpha]_D^{25} +9.1$ (c 0.09, MeOH). The molecular formula was determined as $C_{30}H_{36}O_4$ by HREIMS $[M]^+$ m/z 460.2610 in conjunction with NMR data. The IR spectrum exhibited bands due to hydroxy groups at ν_{\max} 3705, 3416 and a carbonyl function at ν_{\max} 1602. The UV bands at λ_{\max} 203, 241, 309, and 407 nm were similar to those of harunmadagascarins A and B,¹² suggesting that **1** is an anthrone derivative. In the ¹H NMR spectrum (Table 1), the deshielded resonances at δ_H 17.00 and 10.03 were assigned to two hydroxy groups located in the harunganin type structure at C-9 and C-8, respectively.^{13,14} The aromatic proton region displayed one sharp proton resonance at δ 7.09 and one set of AX resonances at δ 6.95 and 6.66 (1H each, d, $J = 0.9$) assignable to a m -coupled proton. These resonances were assigned to H-10, H-5, and H-7, respectively, by comparison with the NMR data of harunmadagascarins A and B.¹² The three-proton resonance at δ 2.40 could be assigned to the C-6 aromatic methyl. The ¹H NMR spectrum (Table 1) also showed resonances due to two prenyl groups [δ 1.42, 1.46,

1.48, 1.50 (3H each, s); 2.86 (4H, $J = 6.2$); 4.51 (4H, t, $J = 6.2$)] attached to a saturated carbon. This was confirmed in the ¹³C NMR spectra (Table 2), which displayed the resonances of two methylene (δ 40.1 and 40.7), two methine (δ 118.2 and 118.3), and four methyl (δ 25.6, 25.6, 18.1, and 18.2) carbons. Furthermore, resonances consistent with an α,α,β -trimethylfuran ring { δ 3.04 (1H, q, $J = 6.9$ Hz), 1.37 and 1.32 (3H each, s), 1.14 (3H, d, $J = 6.9$)} were observed. The presence of this group was supported by the carbon resonances at δ 93.3 (oxygenated sp³ carbon), 43.1 (methine carbon), 28.6, 22.0 (geminal dimethyl carbons), and 14.8 (methyl carbon). These substituents were located on the anthrone skeleton according to HMBC experiments (Figure 3). The long-range correlation from H-18 (δ 1.14) to C-1 (δ 188.6) and C-3 (δ 178.9) suggested the location of an α,α,β -trimethylfurano moiety at C-2–C-3. Furthermore, the C-6 methyl group (δ 2.40) displayed cross-peaks with C-7 (δ 111.7), C-6 (δ 140.3), and C-5 (δ 117.8), and H-5 (δ 6.66) showed a cross-peak with C-10 (δ 115.2). Additionally, H-10 (δ 7.09) showed ³ J correlation with C-4 (δ 46.7), which in turn correlated with H-12 and H-12' (δ 4.51). Thus, the two prenyl groups were located at C-4. From the above evidence, the structure of harunmadagascarin C (**1**) was determined as (+)-8,9-dihydroxy-4,4-bis(3,3-dimethylallyl)-6-methyl-2,3-(2,2,3-trimethylfurano)anthrone. This is the first report of an α,α,β -trimethylfuran anthranoid annulated ring in a natural product, and the furan substitution pattern was found only in some synthetic compounds as an abnormal Claisen rearrangement product of α,α -dimethylallyl ethers.^{15–17} The absolute configuration of C-18 could not be established.

Anthranoid **2**, named harunmadagascarin D, was isolated as optically active yellow crystals, $[\alpha]_D^{25} -64.2$ [c 0.14, (CH₃)₂CO]. Its molecular formula $C_{30}H_{36}O_5$ was deduced from the HREIMS, which showed the molecular ion peak $[M]^+$ at m/z 476.2146. The IR bands appeared at ν_{\max} 2966 (hydroxy), 1721 (carbonyl), and 1628 (conjugated carbonyl) cm⁻¹. The absorption bands observed in its UV spectrum at λ_{\max} 230, 281, 389, and 423 were similar to those of kengaquinone, a 1,4-anthraquinone isolated recently from the same plant.¹³ The presence of the two carbonyl groups was confirmed by the ¹³C NMR spectrum, which showed resonances at δ 201.6 and 207.2. The ¹H NMR spectrum showed one singlet for an aromatic methyl group at δ 2.38, two hydroxy proton resonances at δ 15.77 and 9.63, and the presence of two γ,γ -dimethylallyl moieties. The two hydroxy groups were located at C-9 and C-8 and the methyl group at C-6 on the basis of biogenetic considerations^{12,13} and by comparison of the spectroscopic data with those reported for similar compounds.^{8,9,12} The ¹³C NMR spectrum

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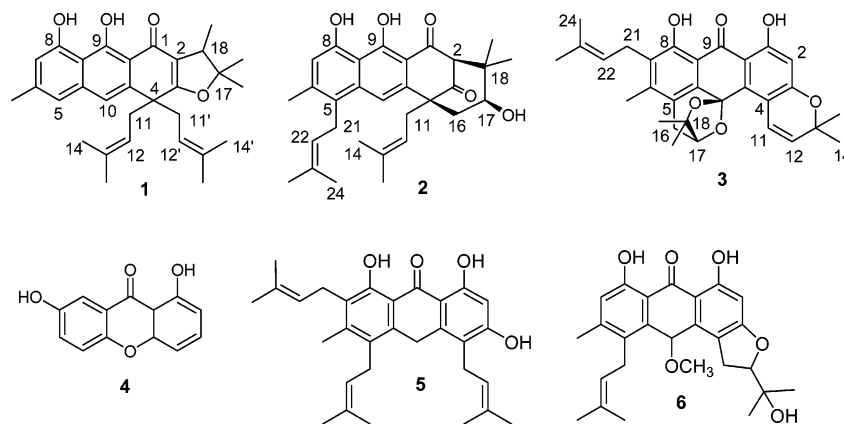


Figure 1. Structures of compounds **1–6** isolated from *H. madagascariensis*.

Table 1. ^1H NMR Data of Harunmadagascarins C (**1**) and D (**2**) and Kenganthranol D (**3**) (δ ppm, 400 MHz, CDCl_3)

H	1	2	3
1-C=O (OH)			13.07 (1H, s)
2		3.14 (1H, s)	6.44 (1H, s)
5	6.66 (1H, d, $J = 0.9$)		
7	6.95 (1H, d, $J = 0.9$)	6.74 (1H, s)	
8-OH	10.03 (1H, s)	9.63 (1H, s)	12.40 (1H, s)
9-OH	17.00 (1H, s)	15.77 (1H, s)	
10	7.09 (1H, s)	7.23 (1H, s)	
11	2.86 (2H, d, $J = 6.2$)	2.83 (1H, dd, $J = 6.4, 15.5$) 2.64 (1H, dd, $J = 5.9, 15.5$)	7.63 (1H, d, $J = 10.5$)
11'	2.86 (2H, d, $J = 6.2$)		
12	4.51 (1H, t, $J = 6.2$)	4.94 (1H, dd, $J = 5.9, 6.4$)	5.67 (1H, d, $J = 10.5$)
12'	4.51 (1H, t, $J = 6.2$)		
14 (pro-Z)	1.42 (3H, s)	1.72 (3H, s)	1.44 (3H, s) ^a
14' (pro-Z)	1.46 (3H, s)		
15 (pro-E)	1.48 (3H, s) ^a	1.60 (3H, s)	1.42 (3H, s) ^a
15' (pro-E)	1.50 (3H, s) ^a		
16		2.54 (1H, dd, $J = 4.2, 14.8$) 2.18 (1H, dd, $J = 1.6, 14.8$) 3.61 (1H, dd, $J = 1.6, 4.2$)	3.13 (1H, dd, $J = 5.0, 16.7$) 2.70 (1H, dd, $J = 0.8, 16.7$) 4.75 (1H, dd, $J = 0.8, 5.0$)
17			
18	3.04 (1H, q, $J = 6.9$)		
19	1.37 (3H, s) ^b	1.29 (3H, s) ^b	1.45 (3H, s) ^b
20	1.32 (3H, s) ^b	1.04 (3H, s) ^b	1.19 (3H, s) ^b
CH ₃ -18	1.14 (3H, d, $J = 6.9$)		
CH ₃ -6	2.40 (3H, s)	2.38 (3H, s)	2.19 (3H, s)
21		3.52 (2H, d, $J = 6.1$)	3.43 (1H, dd, $J = 6.5, 11.8$) 3.39 (1H, dd, $J = 5.4, 11.8$) 5.03 (1H, dd, $J = 5.4, 6.5$)
22		4.94 (1H, t, $J = 6.1$)	
24 (pro-Z)		1.82 (3H, s)	1.78 (3H, s)
25 (pro-E)		1.66 (3H, s)	1.67 (3H, s)

^{a, b}Resonances with the same superscripts in the same column may be interchanged.

displayed resonances that corroborated the presence of two γ,γ -dimethylallyl moieties at δ 27.3, 32.7 (methylene carbons), 122.7, 120.0 (methine carbons), 131.6, 133.3 (quaternary sp^2 carbons), and 18.0, 18.1, 25.6, 25.8 (methyl carbons). In the HMBC spectrum, one of the protons resonating at δ 4.94 (H-22) showed a correlation with C-5 (δ 126.3), which in turn showed cross-peaks with H-7 (δ 6.74) and CH₃-6 (δ 2.38). The proton resonating at δ 4.94 (H-12) displayed a correlation with the carbon resonance at δ 52.5 (C-4). These observations indicated the attachment of the two prenyl groups at C-4 and C-5. The proton resonating at δ 7.23 (H-10) was correlated with the carbon resonance at δ 52.5 (C-4), supporting the location of a prenyl moiety at C-4. The ^1H NMR spectrum showed a set of resonances corresponding to fragment **A** (Figure 2) at δ 1.29 and 1.04 (two methyl groups bonded to nonprotonated aliphatic carbons), 3.61 (an oxymethine), and 2.54 and 2.18 (a methylene group).

This finding was supported by the appearance in the ^{13}C NMR spectrum (Table 2) of resonances at δ 23.8, 26.6 ($2 \times \text{CH}_3$), 73.9 (CHOH), 48.9 (quaternary carbon), and 47.7 (CH₂) and the presence of characteristic fragments at m/z 390 [$\text{M} - \text{C}_5\text{H}_{10}\text{O}$] and 324 [$\text{M} - \text{C}_5\text{H}_9 - \text{C}_5\text{H}_{10}\text{O}$] in the EIMS. In the HMBC spectrum, the two

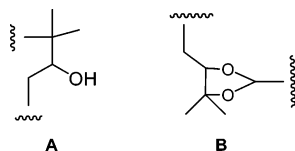
resonances at δ 1.29 and 1.04 (methyl groups) displayed cross-peaks with the carbon resonances at δ 48.9 (C-18), 73.9 (C-17), and 70.4 (C-2). The proton attached to the latter carbon also showed 2J correlations with the quaternary carbon at δ 48.9 (C-18) and the two carbonyl groups at δ 201.6 (C-1) and 207.2 (C-3) and 3J correlations with the resonances at δ 73.9 (C-17), 111.4 (C-9a), and 52.5 (C-4), demonstrating the attachment of fragment **A** at C-2 and C-4. Thus, the observed NMR resonances are characteristic of compound **2**. The structure assignment was confirmed by its HMBC spectrum and by comparison of its ^1H and ^{13}C NMR data with those reported for harunganin.¹⁴ The assignment of the relative configuration as shown in structure **2** (Figure 1) was based on the fact that the "meta" bridge on C-2 and C-4 of the three carbons C-16, C-17, and C-18 had to be on the same side. In addition, the β -orientation of 17-OH was unambiguously established on the basis of the coupling constants $J_{16,17} = 1.6$ and 4.2 Hz. The absence of any *trans*-diaxial coupling placed 17-H in an equatorial and hence 17-OH in an axial position.

Interestingly, the prenyl side chain at C-4 is annulated *ansa*-like onto the anthracene moiety. Possibly, the cyclization was initiated by attack of the nucleophilic C-2 on an epoxidized prenyl side chain.

Table 2. ^{13}C NMR Data of Harunmadagascariens C (**1**) (100 MHz) and D (**2**) (125 MHz) and Kenganthranol D (**3**) (100 MHz) (δ ppm, CDCl_3)

C	1	2	3
1	188.6	201.6	165.5
2	118.1	70.4	106.6
3	178.9	207.2	161.9
4	46.7	52.5	113.8
4a	140.2	138.9	132.2
5	117.8	126.3	120.1
6	140.3	142.0	144.1
7	111.7	114.4	129.0
8	157.2	155.9	158.8
8a	111.2	115.5	109.7
9	162.9	164.4	190.7
9a	109.7	111.4	110.2
10	115.2	112.6	101.2
10a	138.0	136.7	136.2
11	40.7 ^a	32.7	120.3
11'	40.1 ^a		
12	118.2 ^b	120.0	129.2
12'	118.3 ^b		
13	134.2	133.3	76.5
13'	134.2		
14 (pro-Z)	18.1 ^c	18.1	28.1 ^a
14' (pro-Z)	18.2 ^c		
15 (pro-E)	25.6	25.8	28.3 ^a
15' (pro-E)	25.6		
16		47.7	29.0
17	93.3	73.9	82.5
18	43.1	48.9	81.5
19	22.0 ^d	23.8 ^a	24.5 ^b
20	28.6 ^d	26.6 ^a	29.9 ^b
CH ₃ -18	14.8		
CH ₃ -6	21.9	20.9	15.5
21		27.3	24.8
22		122.7	121.6
23		131.6	132.2
24 (pro-Z)		18.0	17.9
25 (pro-E)		25.6	25.7

^{a-d}Resonances with the same superscripts in the same column may be interchanged.

**Figure 2.** Fragments **A** (compound **2**) and **B** (compound **3**).

The optically active ($[\alpha]_{\text{D}}^{25} +21.4$) anthranoid **3**, named kenganthranol D, was isolated as yellow crystals, mp 163 °C. From HREIMS data (m/z 488.2146; calc 488.2198), its molecular formula was found to be $\text{C}_{30}\text{H}_{32}\text{O}_6$. The IR spectrum of **3** indicated the presence of hydroxy (3320 cm^{-1}) and conjugated carbonyl (1631 cm^{-1}) groups. Its ^1H NMR spectrum showed the presence of two deshielded hydroxy groups at δ 13.07 and 12.40. The ^{13}C NMR spectrum exhibited a resonance of only one carbonyl group at δ 190.7, suggesting a 1,8-dihydroxyanthronoid skeleton.^{8,12–14} Additionally, the ^1H NMR spectrum showed resonances that could be assigned to an aromatic methyl group [δ 2.19 (3H, s)], one prenyl group [δ 5.03 (1H, dd, $J = 5.4, 6.5$), 3.43 (1H, dd, $J = 6.5, 11.8$), 3.39 (1H, dd, $J = 5.4, 11.8$), 1.78 and 1.67 (3H each, s)], and a 2,2-dimethylpyran moiety [δ 1.44 and 1.42 (3H each, s), and δ 5.67 and 7.63 (1H each, d, $J = 10.5$)]. The aromatic methyl group resides at C-6 according to HMBC analysis and in agreement with biosynthesis.¹⁵ In the HMBC experiment, the long-range correlation (3J) observed between the H-6 methylene proton resonance (δ 3.39) and C-6 (δ 144.1) and C-8 (δ 109.7) suggested the location of a prenyl moiety at C-7. This was confirmed by a resonance at δ 12.40 (hydroxy group), which exhibited correlations with three carbons including C-8 (δ 109.7). The BB and DEPT spectra revealed the

presence of 30 resonances, including seven methyls, five methines, two methylenes, and 16 quaternary carbons, among them resonances for an oxymethine (δ 82.5), a quaternary oxygenated carbon (δ 81.5), and an acetal carbon atom at δ 101.2. The HMBC spectrum showed that the two C-18 methyl proton resonances (δ 1.45 and 1.19) correlated with the oxymethine (δ 82.5, C-17) and the quaternary oxygenated carbon (δ 81.5, C-18), and H-17 at δ 4.75 (oxymethine) exhibited correlations with C-10 at δ 101.2 (acetal carbon). These observations were conclusive for the deduction of fragment **B** (Figure 2). This fragment was confirmed by the characteristic ions at m/z 101 [$\text{C}_5\text{H}_9\text{O}_2$] and 387 [$\text{M} - \text{C}_5\text{H}_9\text{O}_2$] in the EIMS. Furthermore, in the HMBC spectrum the methylene proton resonances at δ 3.13 and 2.70 showed 2J and 3J correlations with the oxymethine carbon at δ 82.5 and the quaternary oxygenated carbon at δ 81.5, respectively, and the proton resonance at δ 4.75 (oxymethine, H-17) displayed a cross-peak with C-5 (δ 120.1). In the $^1\text{H}-^1\text{H}$ COSY spectrum, H-17 at δ 4.75 exhibited correlations with the two resonances at δ 3.13 and 2.70 attributed to the methylene protons at C-16. These findings were in agreement with the junction of fragment **B** at C-5 and C-10. Inspection of molecular models led to the conclusion that the 10,17 oxygen bridge had to be *cis*, and structure **3** in Figure 1 shows the relative configuration. The remaining 2,2-dimethylpyran fragment was located at C-3, C-4 on the basis of the following observations. In the HMBC spectrum, correlations were observed from the hydroxy resonance at δ 13.07 (OH-1) with C-1 (δ 165.5), C-9a (δ 110.2), and C-2 (δ 106.6). The last carbon was found to be attached to one aromatic proton in the HMQC spectrum, suggesting the location of a 2,2-dimethylpyran group at C-3 and C-4. Further confirmation was the correlation of the vinyl proton resonance at δ 7.63 with the carbon resonances at δ 161.9 (C-3), 113.8 (C-4), and 132.2 (C-4a). On the basis of the above spectroscopic data and by comparison with previously reported anthranoids,^{6–14} the structure of kenganthranol D was fully assigned. Quinone acetals are extremely rare natural products. The anthraquinone acetal found in kenganthranol D is the first report of such a functional group in a secondary plant metabolite. However, a related compound was isolated during the fermentation process of *Aspergillus*, as described in a Japanese patent.¹⁷

Apart from compound **1**, the isolated compounds were tested for antibacterial, antifungal, and algicidal activities (rough correlation with herbicidal activities) (Table 3). Compound **4** showed strong activity in all three tests. Compounds **2** and **6** showed antibacterial activity against the Gram-positive bacteria *Bacillus megaterium*. Compounds **3** and **5** were not active in these tests.

In summary, the structures of three new antibacterial anthranoids are presented. The prenyl side chains are annulated in four different modes, resulting in tetra- and hexacyclic anthranoid ring systems.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter using a 10 cm cell. UV spectra were obtained on a Hitachi UV 3200 spectrophotometer, and IR spectra were recorded on a JASCO 302-A spectrophotometer in KBr disks. Mass spectra and HREIMS were recorded on a JEOL HX 110 mass spectrometer. 1D and 2D NMR spectra were obtained with Bruker Avance AMX 400 and AMX 500 MHz NMR spectrometers, with TMS as an internal standard. The chemical shifts are given in ppm (δ), and coupling constants (J) are in Hz. Silica gel [Merck, Kieselgel 60 (0.070–0.230 mm) and 0.023–0.040 mm] was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm and 1 mm) were used for TLC and preparative TLC analysis. Spots were visualized under UV light (254 and 366 nm) and by spraying with ceric sulfate followed by heating.

Plant Material. The leaves (1.45 kg) of *H. madagascariensis* were collected (March 2004) from the Keng district, Bandjoun, West Province of Cameroon. Authentication was performed by comparison

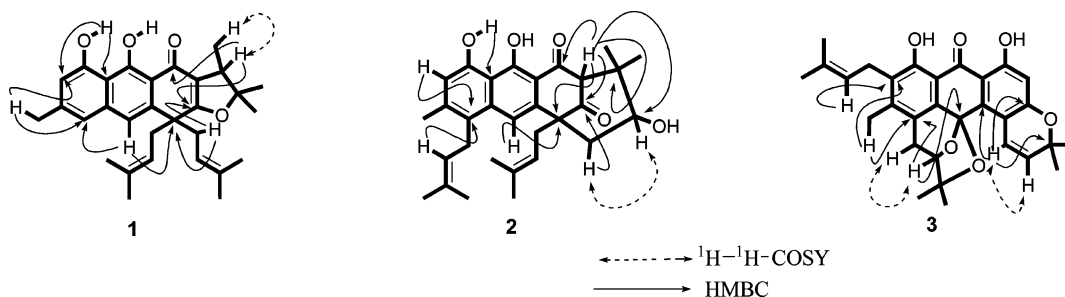


Figure 3. Selected ^1H – ^1H COSY and HMBC correlations for compounds 1–3.

Table 3. Biological Activity of the Pure Compounds^a

microbial activities	isolated compounds					
		2	3	4	5	6
antibacterial (Bm)	a	–	–	9	–	6
	b	10	–	12	–	10
antifungal (Mb)	a	–	–	–	–	–
	b	–	–	12	–	–
algicidal (Chl)	a	–	–	–	–	–
	b	–	–	10	–	–

^aCompounds 2–6 (50 μL at a concentration of (a) 1 $\mu\text{g}/\mu\text{L}$ and (b) 5 $\mu\text{g}/\mu\text{L}$) were tested in an agar diffusion assay for inhibitions of *Bacillus megaterium* (Bm), *Chlorella fusca* (Chl), and *Microbotryum violaceum* (Mb). The radius of the zone of inhibition was measured in mm. (–): inactive

with herbarium specimens at the National Herbarium, Yaoundé, Cameroon, where a voucher specimen (HNC 32358, SRF/3338) is deposited.

Extraction and Isolation. The pulverized leaves of *H. madagascariensis* were extracted twice with MeOH at room temperature for 48 and 8 h. The extract was concentrated in vacuo to obtain a residue of 210 g. This residue was re-extracted with hexane, and the hexane-soluble portion (18.3 g) was subjected to column chromatography (CC) over a silica gel column (60–120 mesh, 180 g) using hexane with a gradient of EtOAc. The column eluates were monitored by thin-layer chromatography, and similar fractions were combined to give three fractions (A–C). Fraction A (2.1 g) was further purified on a silica gel column (60–120 mesh, 20.0 g) with hexane–EtOAc (2%) as eluant to afford, after PTLT, compounds 5 (16.7 mg) and 1 (4.2 mg). Fraction B (3.0 g) was rechromatographed over a Si gel column (60–120 mesh, 18.0 g) with varying proportions of hexane and EtOAc (0–4%) as eluant. Subfractions 1, 2, and 3 were obtained on elution with 49:1, 48:2, and 47:3 mixtures. Repeated column chromatography of subfraction 1 (2.3 g) afforded compound 2 (7.2 mg) and the known compound 4 (15.4 mg). Subfraction 2 (1.5 g) yielded compound 3 (6.8 mg), and subfraction 3 (2.8 g) yielded compound 6 (18.9 mg). Fraction C (7.5 g) was subjected to further CC using a gradient solvent of hexane–EtOAc (0–5%) to give again compounds 2 (1.5 mg) and 4 (2.7 mg).

Harunmadagascarin C (1): orange, gummy pigment; $[\alpha]_D^{25} +9.1$ (*c* 0.09, MeOH); UV λ_{max} nm (MeOH) (log ϵ) 203 (4.20), 241 (4.31), 309 (4.41), 407 (4.53); IR (KBr) ν_{max} 3705, 3416, 3381, 2966, 2966, 2924, 2856, 1731, 1602, 1445, 1380, 1253, 1109, 770, 522 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS m/z (rel int) 460 (10), 404 (4), 391 (100), 361 (4), 349 (48), 336 (31), 334 (4), 321 (19), 307 (6), 293 (8), 281 (6), 256 (5), 239 (3), 213 (4), 185 (5), 167 (7), 149 (30), 129 (8), 97 (10), 83 (17), 69 (38); HREIMS m/z 460.2610 (calcd for $\text{C}_{30}\text{H}_{36}\text{O}_4$, 460.2613).

Harunmadagascarin D (2): yellow crystals from hexane–EtOAc; mp 197 $^{\circ}\text{C}$; $[\alpha]_D^{25} -64.2$ (*c* 0.14, CH_2COCH_3); UV λ_{max} nm (MeOH) (log ϵ) 230 (4.47), 281 (4.50), 389 (3.63), 423 (3.70); IR (KBr) ν_{max} 3729, 2966, 2917, 1721, 1628, 1443, 1381, 1323, 1146, 856, 671 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS m/z (rel int) 476 (77), 408 (23), 390 (8), 337 (25), 335 (12), 323 (8), (321 (16), 307 (12), 293 (16), 281 (18), 269 (17), 95 (15), 91 (13), 87 (11), 85 (43), 69 (100); HREIMS m/z 476.2560 (calcd for $\text{C}_{30}\text{H}_{36}\text{O}_5$, 476.2562).

Kenganthranol D (3): yellow crystals from hexane–EtOAc; mp 163 $^{\circ}\text{C}$; $[\alpha]_D^{25} +21.4$ (*c* 0.014, CHCl_3); UV λ_{max} nm (MeOH) (log ϵ) 202 (4.88), 413 (4.32); IR (KBr) ν_{max} 3751, 3320, 2929, 2925, 1631, 1597, 1452, 1377, 1303, 1251, 1160, 1149, 1008, 943, 827, 761 cm^{-1} ;

^1H and ^{13}C NMR, see Tables 1 and 2; EIMS m/z (rel int): 488 (60), 473 (100), 430 (29), 415 (88), 387 (9), 371 (5), 345 (5), 332 (5), 186 (5), 101 (7), 69 (2); HREIMS m/z 488.2146 (calcd for $\text{C}_{30}\text{H}_{32}\text{O}_6$, 488.2198).

Bioactivity: Agar Diffusion Assay. The compounds to be tested were dissolved in acetone at concentrations of 1 and 5 $\mu\text{g}/\mu\text{L}$. Fifty microliters of each solution was pipetted onto a sterile filter disk, which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the respective test organism.¹⁸ The disks (9 mm) were air-dried, placed on an inoculated agar plate, and incubated at 28 $^{\circ}\text{C}$ overnight. The test organisms were *B. megaterium* (NB medium), *M. violaceum* (Mb), and *C. fusca* (MPY); the radius of the zone of inhibition was measured in mm.

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References and Notes

- Hutchinson, J.; Dalziel, J. M. *Flora of West Tropical Africa*, Millbank: London, 1954; Vol. 1, Part 1, pp 286–290.
- Hedin, L. *Etude sur la Forêt et les Bois du Cameroun*; Librairie Larousse, 2001; pp 123–124
- Okoli, A. S.; Okeke, M. I.; Iroegbu, C. U.; Ebo, P. U. *Phytother. Res.* **2002**, *16*, 174–179.
- Atindehou, K. K.; Kone, M.; Terreaux, C.; Traore, D.; Hostettmann, K.; Dosso, M. *Phytother. Res.* **2002**, *16*, 497–502.
- Madubunyi, I. I.; Obi, S. K. C.; Nwebube, N. I.; Chime, A. B. *Int. J. Pharmacol.* **1995**, *33*, 129–134.
- Nagem, T. J.; De Jesus Faria, T. *Phytochemistry* **1990**, *29*, 3362–3364.
- Miraglia, M. D. C. M.; Mesquita, A. A. L.; Varejao, M. D. J. C.; Gottlieb, O. R.; Gottlieb, H. E. *Phytochemistry* **1981**, *20*, 2041–2042.
- Monache, F. D.; Ferrari, F.; Bettolo, G. B. M.; Suarez, L. E. C. *Planta Med.* **1980**, *40*, 340–346.
- Buckley, D. G.; Ritchie, E.; Taylor, W. C.; Young, L. M. *Aust. J. Chem.* **1972**, *25*, 843–855.
- Inuma, M.; Hideki, T.; Toshiyuki, T.; Mohammad, A. *Phytochemistry* **1995**, *40*, 267–270.
- Inuma, M.; Tosa, H.; Tanaka, T.; Ito, T.; Yonemori, S.; Chelladurai, V.; Aquil, M.; Takahashi, Y.; Naganawa, H. *Heterocycles* **1996**, *43*, 1521–1527.
- Kouam, S. F.; Ngadjui, B. T.; Krohn, K.; Wafo, P.; Ajaz, A.; Choudhary, M. I. *Phytochemistry* **2005**, *66*, 1174–1179.
- Kouam, S. F.; Khan, S. N.; Krohn, K.; Ngadjui, B. T.; Kapche, G. W. F.; Yapna, D.; Zareem, S.; Amal, M. Y. M.; Choudhary, M. I. *J. Nat. Prod.* **2006**, *69*, 229–233.
- Ritchie, E.; Taylor, W. C. *Tetrahedron Lett.* **1964**, *23*, 1431–1436. Jefferson, A.; Scheinmann, F. *J. Chem. Soc. Sect. C* **1969**, 243–245.
- Melvyn, M.; Alberto, G. *J. Nat. Prod.* **1992**, *55* (3), 372–375
- Raju, K. V. S.; Sudha, K.; Srimannarayan, G. *Ind. J. Chem. Sect. B* **1980**, *19B*, 866–870.
- Guay, V.; Brassard, P. *J. Nat. Prod.* **1986**, *49*, 122–125.
- Taito, N.; Michito, T.; Hirofumi, O.; Tatsuya, S. Agrochemical N-1477 manufacture with Aspergillus. Jpn. Kokai Tokkyo Koho JKXXAF JP 2001261610 A2 20010926, 2001, 15 pp; *Chem. Abstr.* **2001**, *135*, 256198.
- Schulz, B.; Sucker, J.; Aust, H.-J.; Krohn, K.; Ludewig, K.; Jone, P. G.; Doering, D. *Mycol. Res.* **1995**, 1007–1015.