JOURNAL OF NATURAL PRODUCTS

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# Antiplasmodial and Antiproliferative Pseudoguaianolides of *Athroisma proteiforme* from the Madagascar Dry Forest[1]

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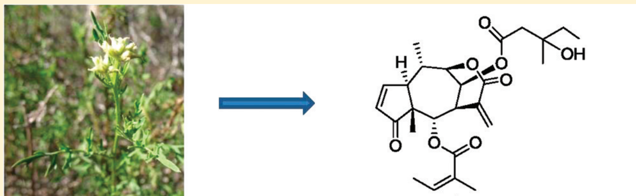
Supporting Information

**ABSTRACT:** Investigation of extracts from the plant *Athroisma proteiforme* (Humbert) Mattf. (Asteraceae) for antimalarial activity led to the isolation of the five new sesquiterpene lactones **1**−**5** together with centaureidin (**6**). The structures of the new compounds were deduced from analyses of physical and spectroscopic data, and the absolute configuration of compound **1** was confirmed by an X-ray crystallographic study. Athrolides C (**3**) and D (**4**) both showed antiplasmodial activities with $IC_{50}$ values of 6.6 (**3**) and 7.2 $\mu$M (**4**) against the HB3 strain and 5.5 (**3**) and 4.2 $\mu$M (**4**) against the Dd2 strain of the malarial parasite *Plasmodium falciparum*. The isolates **1**−**6** also showed antiproliferative activity against A2780 human ovarian cancer cells, with $IC_{50}$ values ranging from 0.4 to 2.5 $\mu$M.



**4**

*Athroisma proteiforme* (photograph by Herisoa Manjakahery)

$IC_{50}$ = 3.9 $\mu$M *P. falciparum* strain Dd2
$IC_{50}$ = 0.57 $\mu$M (A2780 ovarian cancer cells)

In our continuing search for biologically active natural products from tropical rainforests, we obtained an ethanol extract from the aerial parts of a plant identified as *Athroisma proteiforme* (Humbert) Mattf. (Asteraceae) from the Toliara dry forest in southwest Madagascar. The extract exhibited moderate antimalarial activities against HB3 (chloroquine sensitive; CQS) and Dd2 (chloroquine resistant; CQR) *P. falciparum* strains with $IC_{50}$ values of less than 4 $\mu$g/mL to each strain. On the basis of these activities and the paucity of previous phytochemical studies on this genus, *A. proteiforme* was selected for bioassay-guided fractionation to isolate the antiplasmodial components.

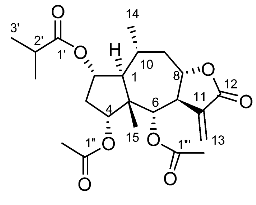
*Athroisma proteiforme* was previously known as *Polycline proteiformis*, and the genus is represented by three endemic species in Madagascar. The only previous phytochemical work on it was the isolation of thymol and a menthene diol from *A. gracile*,[2] and no phytochemical work has been reported on the genus *Polycline*. Previous phytochemical studies of plant species belonging to the family Asteraceae have revealed the presence of antimalarial sesquiterpene lactones[3−7] and flavonoids.[8,9] Among all natural products with antimalarial activity, including alkaloids, terpenoids, flavonoids, limonoids, chalcones, peptides, xanthones, quinones,[2] and coumarins,[10] the sesquiterpene artemisinin from the traditional Chinese medicinal plant *Artemesia annua* (Asteraceae) is one of the most important clinically used antimalarial agents,[4] and it and its derivatives are currently used in artemisinin-based combination therapies (ACTs).[11,12] The probability of isolating additional antimalarial sesquiterpenes thus provided a further incentive to investigate this plant.

## RESULTS AND DISCUSSION

The ethanol extract of the aerial parts of *A. proteiforme* was subjected to liquid−liquid partitioning to give hexanes, $CH_2Cl_2$, and MeOH fractions with $IC_{50}$ values of 4.0, 1.5, and >10 $\mu$g/mL, respectively, against the HB3 strain, and 2.0, 1.0, and 8.0 $\mu$g/mL, respectively, against the Dd2 strain. Fractionation of the most active $CH_2Cl_2$ fraction by C-18 open column and HPLC yielded five new sesquiterpene lactones, designated athrolides A−E (**1**−**5**), as well as the known flavonoid centureidin (**6**). Herein we report the structural elucidation, the antimalarial properties, and the antiproliferative activities of the isolates.

Athrolide A (**1**) was obtained initially as an off-white solid. Its positive ion HR-ESIMS revealed a pseudo molecular ion peak at $m/z$ 437.2176 $[M + H]^+$, corresponding to the molecular formula $C_{23}H_{32}O_8$. The IR spectrum showed strong absorption in the range of 1740−1710 $cm^{-1}$, consistent with the presence of ester and $\alpha,\beta$-unsaturated lactone groups. Its $^1H$ NMR spectrum in $CDCl_3$ showed signals for four methyl singlets at $\delta_H$ 2.10, 2.09, 2.08, and 0.98, three methyl doublets at $\delta_H$ 1.16, 1.16, and 1.05, one vinyl methylene (two doublets at $\delta_H$ 6.27 and 5.65, $J$ = 3.2 Hz, H-13a and H-13b), two pairs of methylenes at $\delta_H$ 2.64 (m, H-3a) and 1.32 (dd, $J$ = 16.3, 2.7 Hz, H-3b) and at $\delta_H$ 2.33 (ddd, $J$ = 12.8, 3.1, 3.1 Hz, H-9a) and 1.55 (m, H-9b), and eight methines ($\delta_H$ 1.88, m (H-10); $\delta_H$ 2.67, m (H-1); $\delta_H$ 2.52, septet, $J$ = 7.0 Hz (H-2′); $\delta_H$ 3.48, m

**Received:** June 14, 2011
**Published:** October 13, 2011



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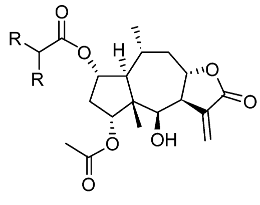
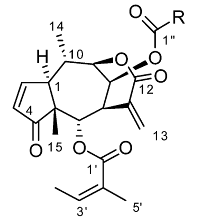
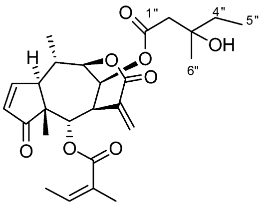


2 R = Me
7 R = H



3 R = $CH_2C(CH_3)_2OH$
8 R = $CH_3$



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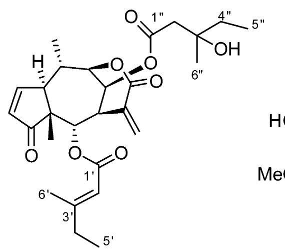

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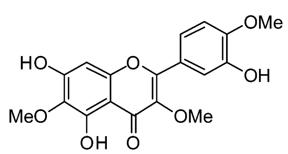

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(H-7); $\delta_H$ 4.11, ddd, $J$ = 12.2, 9.0, 3.5 Hz (H-8); $\delta_H$ 4.95, d, $J$ = 4.9 Hz (H-4); $\delta_H$ 5.03, ddd, $J$ = 8.7, 8.7, 2.7 Hz, (H-2); $\delta_H$ 5.06, d, $J$ = 11 Hz (H-6)). The chemical shift data indicated that four of the methines were on oxygenated carbons (Table 1). The $^{13}C$ NMR spectrum displayed signals for 2 acetoxy groups ($\delta_C$ 169.8 and 20.0 and $\delta_C$ 170.2 and 21.4) and 1 2-methylpropanoyloxy group ($\delta_C$ 176.4, 33.9, 18.8, and 18.7) together with 15 other signals. These signals were assigned by an HMQC spectrum to two quaternary carbons at $\delta_C$ 51.5 (C-5) and 136.2 (C-11), a lactone carbonyl at $\delta_C$ 169.2 (C-12), one quaternary and one secondary methyl at $\delta_C$ 22.4 (C-15) and 20.0 (C-14), two methylenes at $\delta_C$ 37.9 (C-3) and 43.1 (C-9), four oxygen-bearing methines at $\delta_C$ 80.9 (C-8), 79.6 (C-4), 78.1 (C-6), and 75.5 (C-2), three methines at $\delta_C$ 51.3 (C-1), 46.3 (C-7), and 27.2 (C-10), and an vinylic methylene at $\delta_C$ 124.1 (C-13) (Table 1). These data are all interpretable by assignment of a sesquiterpene lactone structure to athrolide A.

Inspection of the $^1H$ NMR data revealed that athrolide A is similar to the known pseudoguaianolides 6-angeloyloxypuchellin[13] and 2-deacetyl-2-isobutyrylchamissonolide.[14] The complete $^1H$ and $^{13}C$ NMR assignments and connectivities were established from a combination of HMQC, COSY, and HMBC data analyses (Figure 1). The COSY spectrum showed correlations that indicated the presence of the spin systems H-4, H-3, H-2, H-1, H-10, H-9, H-8, H-7, H-6, and H-14 and H-2′, H-3′, and H-4′ of the 2-methylpropanoyloxy moiety. In the HMBC spectrum, the correlations from $H_3$-15 to C-4, C-5, C-1, and C-6 as well as the correlations from H-4 to C-1 and to C-5 corroborated the presence of a five-membered ring, fused at C-1 and C-5 with a seven-membered ring. The HMBC correlations from $H_2$-9 to C-1, C-8, C-7, and C-14, from H-6 to C-5, C-7, and C-8, and from $H_2$-13 to C-7 and the lactone carbonyl at C-12 suggested the presence of a $\gamma$-lactone ring, fused at C-7 and C-8. These data indicated that **1** was a pseudoguaianolide.[13,14] The HMBC correlations from two methyl groups ($H_3$-3′ and $H_3$-4′), one septet methine (H-2′), and H-2 to C-1′, H-4 and $H_3$-2″ to C-1″, H-6 and $H_3$-2‴ to C-1‴ indicated the presence of an 2-methylpropanoyl group at C-2, an acetate group at C-4, and another acetate group at C-6. In the NOESY spectrum of **1**, the correlations from H-1 to H-7, H-9b, and $H_3$-14, from $H_3$-15 to H-2, H-4, H-6, H-8, and H-10, and from H-8 to H-6, H-9a, and H-10 suggested that H-1, H-7, and $H_3$-14 were cofacial and that H-2, H-4, H-6, H-8, H-10, and $H_3$-15 were on the opposite face.

In order to determine the absolute configuration, compound **1** was crystallized from MeOH to afford good-quality needle-shaped crystals, and its structure was confirmed by single-crystal X-ray diffraction. An anisotropic displacement ellipsoid drawing is shown in Figure 2. Anomalous dispersion effects confirmed the absolute configuration of **1** to be (1*S*,2*S*,4*R*,5*S*,6*S*,7*R*,8*S*,10*R*)-2-(2-methylpropanoyloxy)-4-acetoxy-6-acetoxyguai-11(13)-en-8,12-olide (**1**, athrolide A).

Athrolide B (**2**) was obtained as an off-white solid. Its positive ion HR-ESIMS revealed a pseudomolecular ion peak at $m/z$ 417.1889 $[M + Na]^+$, corresponding to the molecular formula $C_{21}H_{30}O_7$. Its IR spectrum showed a hydroxyl stretch at 3474 $cm^{-1}$ and strong absorption in the range 1740−1710 $cm^{-1}$. Its $^1H$ NMR spectrum in $CDCl_3$ showed signals for two methyl singlets at $\delta_H$ 2.10 and 0.98, three methyl doublets at $\delta_H$ 1.14, 1.14, and 1.02, three methylene multiplets, two of which were olefinic ($\delta_H$ 2.66, 1.53; 2.42, 1.41; 5.53, 6.40), and eight methines ($\delta_H$ 1.98, 2.24, 2.50, 3.04, 4.41, 4.64, 4.90, and 5.02), four of which were oxygenated (Table 1). Inspection of the $^1H$ and $^{13}C$ NMR data of **2** in $CDCl_3$ showed a close similarity with the data of the previously isolated (1*S*,2*S*,4*R*,5*R*,6*R*,7*S*,8*S*,10*R*)-2,4-diacetoxy-6-hydroxyguai-11(13)-en-8,12-olide (**7**).[15] It differed from **7** in the presence of a 2-methylpropanoyl group at C-2 of **2** compared to an acetate group at C-2 of **7**. HMBC correlations from H-2 to C-1′ and of the methine septet at H-2′ and of $H_3$-3′ and $H_3$-4′ to C-1′, and from H-4 to C-1″ indicated that the 2-methylpropanoyl group was located at C-2 and the acetate group at C-4. In the NOESY spectrum, the correlations from H-1 to H-6, H-7, H-9b, and $H_3$-14, from H-6 to H-7 and H-1, and from $H_3$-15 to H-2, H-4, H-8, and H-10 indicated that H-1, H-6, H-7, and $H_3$-14 were cofacial and that H-2, H-4, H-8, H-10, and $H_3$-15 were on the opposite face. The characteristic UV absorption of an $\alpha,\beta$-unsaturated lactone chromophore was observed at 230 nm.[16] The absolute configuration of **2** was deduced by the comparison of its CD spectrum with that of **1**. The negative Cotton effect for **2** of $[\theta]_{230\ nm} = -1.56 \times 10^3$ was similar to that of **1** ($[\theta]_{230\ nm} = -3.57 \times 10^3$) and enabled assignment of the *S* configuration to C-7 according to the back octant rule.[17] Therefore, athrolide B (**2**) was determined to be (1*S*,2*S*,4*R*,5*R*,6*R*,7*S*,8*S*,10*R*)-2-(2-methylpropanoyloxy)-4-acetoxy-6-hydroxyguai-11(13)-en-8,12-olide.

Athrolide C (**3**) was obtained as an off-white solid. Its positive ion HR-ESIMS revealed a pseudo molecular ion peak at $m/z$ 483.2003 $[M + Na]^+$, corresponding to the molecular formula $C_{25}H_{32}O_8$. The IR spectrum showed a hydroxy absorption (3444 $cm^{-1}$) and strong absorption in the range 1740−1710 $cm^{-1}$, consistent with the presence of ester, keto carbonyl, and lactone groups. Its $^1H$ NMR spectrum in $CDCl_3$ showed signals for three methyl singlets ($\delta_H$ 1.27, 1.26, and 1.07), one methyl doublet ($\delta_H$ 1.41 d, $J$ = 7.2 Hz), two olefinic methyl groups ($\delta_H$ 1.78 m; 1.94 dq, $J$ = 7.3, 1.5 Hz), one singlet

Table 1. $^{1}H$ and $^{13}C$ NMR Data of Athrolides A (1), B (2), C (3), D (4), and E (5)[a]

| | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| position | $^{1}H$ (*J*, Hz) | $^{13}C$ | $^{1}H$ (*J*, Hz) | $^{13}C$ | $^{1}H$ (*J*, Hz) | $^{13}C$ | $^{1}H$ (*J*, Hz) | $^{13}C$ | $^{1}H$ (*J*, Hz) | $^{13}C$ |
| 1 | 2.67 m | 51.3 | 2.24 dd (10.8, 6.8) | 52.8 | 3.19 dm (12.6) | 50.5 | 3.19 dm (12.6) | 50.5 | 3.23 dm (12.8) | 50.2 |
| 2 | 5.03 td (8.7, 2.7) | 75.5 | 5.02 ddd (9.1, 6.9, 2.1) | 77.2 | 7.60 bd (6.0) | 161.0 | 7.60 bd (6.1) | 161.0 | 7.60 bd (6.0) | 161.1 |
| 3 | 2.64 m, 1.32 dd (16.3, 2.7) | 37.9 | 2.66 ddd (16.4, 9.0, 4.8), 1.53 dd (16.5, 2.1) | 38.1 | 6.11 overlapped | 130.3 | 6.11 overlapped | 130.3 | 6.10 m | 130.3 |
| 4 | 4.95 d (4.9), | 79.6 | 4.90 d (4.8) | 82.4 | | 207.7 | | 207.7 | | 207.8 |
| 5 | | 51.5 | | 51.3 | | 55.3 | | 55.3 | | 55.2 |
| 6 | 5.06 d (11.0) | 78.1 | 4.41 bs | 65.0 | 5.34 d (3.7) | 76.0 | 5.34 d (3.7) | 76.0 | 5.31 d (3.7) | 75.3 |
| 7 | 3.48 m | 46.3 | 3.04 m | 52.0 | 3.55 m | 45.5 | 3.56 m | 45.5 | 3.51 m | 45.7 |
| 8 | 4.11 ddd (12.2, 9.0, 3.5) | 80.9 | 4.64 ddd (12.1, 9.1, 3.2) | 75.7 | 5.48 s | 64.7 | 5.48 s | 64.7 | 5.49 s | 65.0 |
| 9 | 2.33 ddd (12.8, 3.1, 3.1), 1.55 m | 43.1 | 2.41 m, 1.41 m | 44.0 | 4.66 s | 88.5 | 4.66 s | 88.5 | 4.66 s | 88.6 |
| 10 | 1.88 m | 27.2 | 1.98 m | 29.0 | 2.43 m | 35.4 | 2.43 m | 35.4 | 2.43 m | 35.4 |
| 11 | | 136.2 | | 136.8 | | 130.8 | | 130.8 | | 131.2 |
| 12 | | 169.2 | | 169.3 | | 162.4 | | 162.4 | | 162.5 |
| 13 | 6.27 d, (3.2), 5.65 d, (3.2) | 124.1 | 6.40 d, (3.6), 5.53 d, (3.6) | 121.0 | 6.80 s, 6.11 s | 134.4 | 6.80 s, 6.11 s | 134.3 | 6.78 s, 6.10 s | 134.1 |
| 14 | 1.05 d (6.8) | 20.0 | 1.02 d (6.6) | 20.9 | 1.41 d (7.2) | 19.2 | 1.41 d (7.2) | 19.2 | 1.41 (7.2) | 19.1 |
| 15 | 1.09 s | 22.4 | 0.98 s | 17.7 | 1.07 s | 18.8 | 1.07 s | 18.8 | 1.05 s | 18.9 |
| 1′ | | 176.4 | | 176.4 | | 166.1 | | 166.1 | | 165.5 |
| 2′ | 2.52 septet (7.0) | 33.9 | 2.50 septet (7.0) | 34.0 | | 127.0 | | 127.0 | 5.53 | 113.3 |
| 3′ | 1.16 d (7.0) | 18.8 | 1.14 d (7.0) | 18.8 | 6.09 m | 139.7 | 6.09 m | 139.6 | | 164.4 |
| 4′ | 1.16 d (7.0) | 18.7 | 1.13 d (7.0) | 18.7 | 1.94 dq (7.3, 1.5) | 15.7 | 1.93 dq (7.2, 1.5) | 15.7 | 2.16 q (7.3) | 33.9 |
| 5′ | | | | | 1.78 m | 20.4 | 1.78 m | 20.4 | 1.05 t (7.3) | 11.8 |
| 6′ | | | | | | | | | 2.15 s | 19.0 |
| 1″ | | 169.8 | | 170.5 | | 171.6 | | 171.7 | | 171.8 |
| 2″ | 2.08 s | 20.0 | 2.10 s | 21.3 | 2.49 s | 46.4 | 2.51 d (15.5) | 44.5 | 2.51 d (15.2) | 44.6 |
| | | | | | | | 2.42 d (15.5) | | 2.43d (15.2) | |
| 3″ | | | | | | 69.2 | | 71.4 | | 71.5 |
| 4″ | | | | | 1.27 s | 29.3 | 1.52 qd (7.5, 2.5) | 34.7 | 1.52 m | 34.7 |
| 5″ | | | | | 1.26 s | 29.2 | 0.90 t (7.5) | 8.2 | 0.90 t (7.5) | 8.3 |
| 6″ | | | | | | | 1.19 s | 26.2 | 1.19 s | 26.2 |
| 1‴ | 2.09 s | 170.2 | | | | | | | | |
| 2‴ | | 21.4 | | | | | | | | |

[a]In $CDCl_3$; $\delta$ (ppm) 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$; multiplicities; *J* values (Hz) in parentheses.

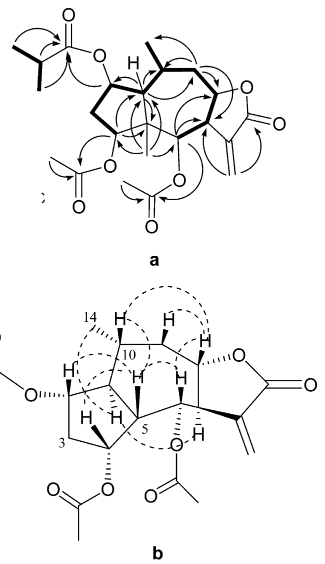


**Figure 1.** Key correlations for **1**: (a) COSY (bold) and HMBC (arrows); (b) NOESY.

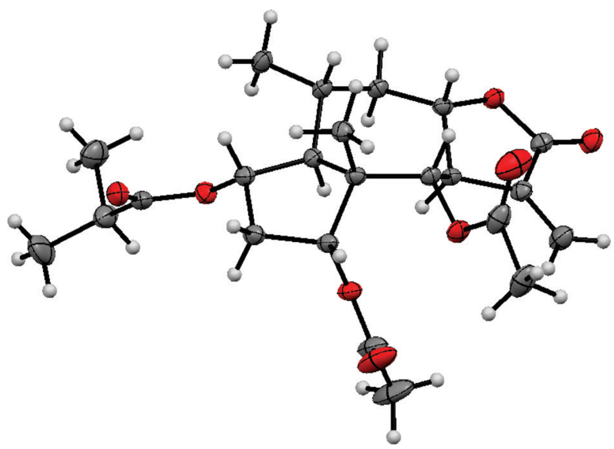

**Figure 2.** Anisotropic displacement ellipsoid drawing of **1**.

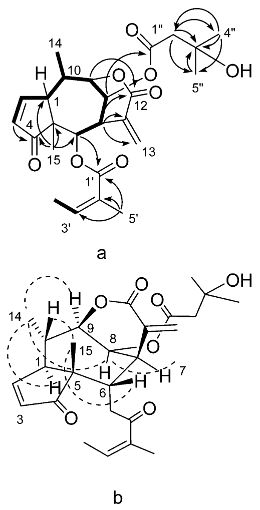


**Figure 3.** Key correlations for **3**: (a) COSY (bold) and HMBC (arrows); NOESY.

methylene ($\delta_H$ 2.49), and three methines ($\delta_H$ 3.55 m, 3.19 m, and 2.43 m). Three oxygenated methines were also observed at $\delta_H$ 5.48 br s, 5.34 (d, $J$ = 3.7 Hz), and $\delta_H$ 4.66 br s. Signals for five olefinic methines were observed at $\delta_H$ 7.60 dd ($J$ = 6.0, 1.6 Hz), 6.80 s, 6.12 s, 6.12 m, and 6.10 m. The $^{13}$C NMR spectrum displayed a set of signals ascribable to a (*Z*)-2-methyl-2-butenoyl (angeloyl) group ($\delta_C$ 166.1, 127.0, 139.7, 15.7, 20.4)[15] and a 3-hydroxy-3-methylbutanoyl group ($\delta_C$ 171.6, 46.4, 69.2, 29.3, 29.2)[18] together with 15 signals of a sesquiterpene lactone (2 quaternary carbons at $\delta_C$ 55.3 and 130.8; 1 lactone carbonyl at $\delta_C$ 162.4, and 1 conjugated carbonyl at $\delta_C$ 207.7; 1 quaternary and 1 secondary methyl ($\delta_C$ 18.8 and 19.2, respectively); 2 olefinic methines ($\delta_C$ 161.0 and 130.3); 3 oxygen-bearing methines ($\delta_C$ 88.5, 76.0, and 64.7); 3 methines ($\delta_C$ 50.5, 45.5, and 50.5); 1 exocyclic methylene ($\delta_C$ 134.4), as indicated by the HMQC spectrum (Table 1)). The complete $^1$H and $^{13}$C NMR assignments and connectivities were established from a combination of COSY, HMQC, and HMBC data (Figure 3). The COSY spectrum showed correlations that indicated the presence of two spin systems: H-3, H-2, H-1, H-10, H-9, H-8, H-7, H-6, and H-14 and H-3′ and H-4′ of the angeloyl moiety. In the HMBC spectrum, the correlations from $H_3$-15 to C-4, C-5, C-1, and C-6 as well as the correlations from H-3 to C-4 and C-5 corroborated the presence of an $\alpha,\beta$-unsaturated cyclopentenone ring fused with a seven-membered ring. The HMBC correlations from H-9 to C-12 at $\delta_C$ 162.4 and H-7 to C-11 at $\delta_C$ 130.8, C-12 at $\delta_C$ 162.4, and C-13 at $\delta_C$ 134.4 suggested the presence of a $\delta$-lactone ring, fused at C-7 and C-9 and substituted at C-8. These data indicated that **3** was a pseudoguaianolide analogue.[19] Inspection of the $^{13}$C NMR data of **3** indicated a close similarity to the data of 1$\alpha$,7$\alpha$,10(H)$\beta$-4-oxo-6$\alpha$-[(*Z*)-2-methyl-2-butenoyloxy]-8$\beta$-acetoxypseudoguaia-2(3),11(13)-dien-9$\beta$,12-olide (**8**), previously isolated from *Hymenoxys ivesiana*.[19] These comparisons indicated that **3** differed from **8** only in the nature of the ester substituent at C-8. The locations of the (*Z*)-2-methyl-2-butenoate (angelate) group at C-6 and the 3-hydroxy-3-methyl butanoate group at C-8 were substantiated by the observation of HMBC cross peaks from H-6 to C-1′, H-3′ to C-1′, H-4′ to C-3′ and C-2′, and H-5′ to C-1′, C-2′, and C-3′ and from H-8 to C-1″, $H_2$-2″ to C-1″, C-3″, C-4″, and C-5″, $H_3$-4″ to C-2″, C-3″, and C-5″, and $H_3$-5″ to C-2″, C-3″, and C-4″.

NOESY correlations of **3** from $H_3$-15 to H-10 and H-6, and $H_3$-14 to H-1, H-9, and H-8, as well as the cross peaks arising from H-7 to H-8 suggested that H-1, H-7, H-8, H-9, and $H_3$-14 were cofacial and that H-6, H-10, and $H_3$-15 were on the opposite face. Therefore, athrolide C (**3**) was determined to be 1*S**,7*R**,10(H)*R**-4-oxo-6*S**-[(*Z*)-2-methyl-2-butenoyloxy]-8*S**-(3-hydroxy-3-methyl-butanoyloxy)pseudoguaia-2(3),11-(13)-dien-9*R**,12-olide.

Athrolide D (**4**) was obtained as an off-white solid. Its positive ion HR-ESIMS revealed a pseudo molecular ion peak at $m/z$ 497.2143 $[M + Na]^+$, corresponding to the molecular formula $C_{26}H_{34}O_8$. Its IR spectrum and $^1$H NMR data were similar to those of athrolide C (**3**). The only significant difference was the presence of a set of signals at $\delta_H$ 2.51 (1H, d, $J$ = 15.5 Hz), 2.42 (1H, d, $J$ = 15.5 Hz), 1.52 (2H, qd, $J$ = 7.5, 2.6 Hz), 1.19 (3H, s), and 0.90 (3H, t, $J$ = 7.5 Hz) instead of those for the 3-hydroxy-3-methylbutanoate group of **3**. The $^{13}$C NMR spectrum displayed signals ascribable to a 3-hydroxy-3-methylpentanoate group[20] ($\delta_C$ 171.7, 71.4, 44.5, 34.7, 26.2, and 8.2). The HMBC correlations from H-8 to C-1″, H-2″ to C-1″ and C-3″, H-4″ to C-2″, C-3″, C-5″, and C-6″, H-5″ to C-3″, and H-6″ to C-2″ and C-3″ as well as the COSY cross peak from H-5″ and H-6″ confirmed the presence of a 3-hydroxy-3-

methylpentanoate group and its location at C-8. The NOESY spectrum of **4** showed correlations similar to those observed for **3**. The correlations from $H_3$-15 to H-10 and H-6 and $H_3$-14 to H-1, H-9, and H-8, as well as the correlation from H-7 to H-8 were observed. Thus, the structure of **4** was assigned as 1*S**,7*R**,10(H)*R**-4-oxo-6*S**-[(*Z*)-2-methyl-2-butenoyloxy]-8*S**-(3-hydroxy-3-methylpentanoyloxy)pseudoguaia-2(3),11-(13)-dien-9*R**,12-olide.

Athrolide E (**5**) was obtained as an off-white solid. Its positive ion HR-ESIMS revealed a pseudo molecular ion peak at *m/z* 489.2496 $[M + H]^+$, corresponding to the molecular formula $C_{27}H_{36}O_8$. Its IR spectrum was similar to those of athrolides C (**3**) and D (**4**). The similarity of its $^1H$ and $^{13}C$ NMR spectroscopic data to those of **4** (Table 1) suggested that **5** was a closely related pseudoguaianolide analogue. The presence of an (*E*)-3-methyl-2-pentenoate group at C-6 was indicated by the observation of a set of signals at $\delta_H$ 5.53 (1H, s), 2.16 (2H, q, $J$ = 7.3 Hz), 2.15 (3H, s), and 1.05 (3H, t, $J$ = 7.3 Hz) in the $^1H$ NMR spectrum, and the corresponding $^{13}C$ NMR data ($\delta_C$ 165.5, 164.4, 113.3, 33.9, 19.0, and 11.8) supported this conclusion.[21] The HMBC correlations from H-6 to C-1′, H-2′ to C-1′ and C-3′, H-4′ to C-2′, C-3′, C-5′, and C-6′, H-5′ to C-3′ and C-4′, and H-6′ to C-2′, C-3′, and C-4′ as well as the COSY cross peak between H-4′ and H-5′ confirmed the location of the (*E*)-3-methyl-2-pentenoate group at C-6. A NOESY correlation from H-2′ to H-4′ assigned the configuration of the double bond as *E*. Finally, the NOESY correlations of the ring protons observed in **5** were similar to those observed in **3** and **4**. Clear correlations from $H_3$-15 to H-10 and H-6, from $H_3$-14 to H-1, H-9, and H-8, and from H-7 to H-8 of **5** confirmed its configuration to be the same as that of **4**. Thus, the structure of **5** was concluded to be 1*S**,7*R**,10(H)*R**-4-oxo-6*S**-(*E*)-3-methyl-2-pentenoyloxy-8*S**-(3-hydroxy-3-methylpentanoyloxy)-pseudoguaia-2(3),11(13)-dien-9*R**,12-olide.

The structure of the known compound **6** was determined to be centaureidin by comparison of its MS and $^{13}C$ NMR data with literature data.[22]

Athrolides A (**1**), C (**3**), D (**4**), and E (**5**) were tested for their antimalarial activities against the drug-sensitive HB3 and drug-resistant Dd2 strains of *P. falciparum* (Table 2); a lack of material prevented the assay of athrolide B (**2**). Athrolide D (**4**) showed the strongest activities against the drug-resistant Dd2 strain with an $IC_{50}$ value of 4.2 $\mu$M, while athrolide C (**3**) had similar activity ($IC_{50}$ 5.5 $\mu$M) against the same strain. Interestingly, both compounds were less potent against the drug-sensitive HB3 strain, with $IC_{50}$ values of 6.6 and 7.2 $\mu$M, respectively. These potencies are less than those obtained for the partially purified fractions described earlier, but no other active materials could be obtained. It is possible that the original extract contained a highly active but unstable compound, but it is also possible that the preliminary assays, which were obtained over a limited dose range, indicated potencies erroneously higher than the true values.

**Table 2. Antiplasmodial and Antiproliferative Data for Athrolides A–E (1–5)**

| | antiplasmodial activity ($IC_{50}$, $\mu$M)[a] | | | antiproliferative activity ($IC_{50}$, $\mu$M)[b] |
|---|---|---|---|---|
| compd | HB3 | Dd2 | $R_f$[c] | A2780 |
| **1** | 34.3 ± 0.3 | 32.7 ± 0.3 | 1.0 | 2.1 ± 0.2 |
| **2** | ND[d] | ND | | 2.5 ± 0.12 |
| **3** | 6.6 ± 0.1 | 5.5 ± 0.03 | 0.8 | 0.57 ± 0.05 |
| **4** | 7.2 ± 0.2 | 4.2 ± 0.7 | 1.7 | 0.38 ± 0.02 |
| **5** | 16.0 ± 0.3 | 11.7 ± 0.3 | 0.7 | 1.9 ± 0.2 |
| chloroquine | 0.0147 ± 0.0007 | 0.224 ± 0.009 | | ND |
| paclitaxel | ND | ND | | 0.017 ± 0.006 |

[a]Data ± standard error of the mean. [b]Data ± standard deviation. [c]Resistance factor. [d]ND = not determined.

The antiproliferative activities of compounds **1**–**6** were also evaluated against the A2780 human ovarian cancer cell line. Athrolides A–E had $IC_{50}$ values of 2.1, 2.5, 0.57, 0.38, and 1.9 $\mu$M, respectively, against this cell line, while centaureidin was also weakly active with an $IC_{50}$ value of 3.9 $\mu$M. The fact that athrolides C and D were more potent toward the drug-resistant Dd2 line than toward the drug-sensitive HB3 line is an interesting observation, but the fact that their antiproliferative $IC_{50}$ values are lower than their antimalarial $IC_{50}$ values suggests that these compounds are not likely to be useful lead compounds because of potential toxicity concerns. The fact that the most potent antiproliferative compounds **3** and **4** were also the most potent antimalarial compounds also suggests that it will prove difficult to separate these two activities in this class of compounds.

## ■ EXPERIMENTAL SECTION

**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. CD analyses were performed on a JASCO J-810 spectropolarimeter with a 1.0 cm cell in MeOH. NMR spectra were recorded in $CDCl_3$ on JEOL Eclipse 500 and Bruker 600 spectrometers. The chemical shifts are given in $\delta$ (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 $\mu$m, 250 × 10 mm).

**Plant Material.** The aerial parts of *Athroisma proteiforme* (Humbert) Mattf. (formerly *Polycline proteiformis* Humbert) (Asteraceae) were collected on April 24th, 1998, near Toliara, Madagascar, at coordinates 23°24′30″S 043°46′40″E and an elevation of 47 m. This aromatic herbaceous plant can grow up to 60 cm in height and bears white flowers. Voucher specimens have been deposited at the Smithsonian Institution, Washington, DC, at the Missouri Botanical Garden, and at the herbarium of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar, under voucher number Richard Randrianaivo 197.

**Extraction and Isolation.** Dried aerial parts of *A. proteiforme* (approximately 500 g) were ground in a hammer mill and then extracted with EtOH by percolation for 24 h at room temperature to give the crude extract N110635 (5 g), 3 g of which was shipped to Virginia Polytechnic Institute and State University for bioassay-guided isolation. The extract N110635 ($IC_{50}$: 1.9 $\mu$g/mL (HB3), 1.6 $\mu$g/mL (Dd2)) (2 g) was suspended in aqueous MeOH ($MeOH/H_2O$ 9/1, 100 mL) and extracted with hexanes (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 hexanes extract was evaporated in vacuo to leave 186.5 mg with $IC_{50}$ values of 4.0 (HB3) and 2.0 $\mu$g/mL (Dd2). The residue from the $CH_2Cl_2$ extract (470.1 mg) had $IC_{50}$ values of 1.5 (HB3) and <1.0 $\mu$g/mL (Dd2). The aqueous MeOH extract (1.392 g) was less active, with $IC_{50}$ values of >10.0 (HB3) and 8.0 (Dd2). The $CH_2Cl_2$ extract was selected for fractionation, and a separation on a C18 open column gave the four fractions I–IV (155.7, 149.7, 54.2, and 19.3 mg). $IC_{50}$ values ($\mu$g/mL) were as follows: fractions I, >5.0 (HB3) and >5.0 (Dd2); fraction II, 0.45 (HB3), 0.42 (Dd2); fraction III, 5.0 (HB3), 3.5 (Dd2); fraction IV, >5.0 (HB3), > 5.0 (Dd2). Fraction II was selected for further separation by C-18 preparative HPLC (60% $CH_3CN/H_2O$). Compounds **1** (2.2 mg, $t_R$ = 22.1 min), **2** (2.2 mg, $t_R$ = 14.6 min), **3** (2.3 mg, $t_R$ = 13.2 min), **4** (2.9 mg, $t_R$ = 16.5 min), **5** (1.1 mg, $t_R$ = 20.2 min), and **6** (2.3 mg, $t_R$ = 10.0 min) were isolated as the major components of this fraction.

**Bioassays.** Antiplasmodial assays were performed against the chloroquine-sensitive HB3 strain and the chloroquine-resistant Dd2 strain of *P. falciparum* at Georgetown University. The assay utilized the previously reported protocol[23] with minor modifications. Typically, the original dried extract was dissolved in DMSO to give stock solutions. Further dilutions of these stock solutions were performed using complete media, finally resulting in working stocks. Samples of the working stock solutions (100 $\mu$L) were transferred into 96-well plates which were prewarmed to 37 °C prior to the addition of the cultures. Sorbitol synchronized cultures were utilized for the assays with >95% of the parasites in the ring stage. Usually, cultures were diluted to give a working stock of 2% parasitemia and 4% hematocrit and 100 $\mu$L was transferred into each drug-preloaded well (final 1% parasitemia and 2% hematocrit). hematocrit and 0.5% parasitemia). The plates were transferred to an airtight chamber which was gassed (90% N, 5% $O_2$, 5% $CO_2$ gas mixture) and incubated at 37 °C. After 72 h, 50 $\mu$L of 10× SYBR Green I dye (diluted using complete media from a 10 000× DMSO stock) was added, the plates were incubated for an additional 1 h at 37 °C to allow DNA intercalation, and fluorescence was measured at 530 nm (490 nm excitation) using a Spectra GeminiEM plate reader (Molecular Devices). $IC_{50}$ values from assays done in triplicate were averaged and are shown ± SEM. In these assays, chloroquine (CQ) was included as a positive control. For $IC_{50}$ calculations, data analysis was performed using Sigma Plot 10.0 software after downloading data in Excel format.[23]

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[24] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[25]

*Athrolide A, (1S,2S,4R,5S,6S,7R,8S,10R)-2-(2-methylpropanoyloxy)-4-acetoxy-6-acetoxyguai-11(13)-en-8,12-olide (**1**):* white solid; $[\alpha]_D^{23}$ = −53° (*c* 0.1, $CHCl_3$); CD $[\theta]_{230}$ = −3570 (MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 211 nm (4.0); IR $\nu_{max}$ 3463, 2965, 1733, 1464, 1375, 1258, 1156, 1052, 1018 $cm^{-1}$. $^1$H NMR (500 MHz, $CDCl_3$) and $^{13}$C NMR (125 MHz, $CDCl_3$), see Table 1; HR-ESIMS *m/z* 437.2176 $[M + H]^+$ (calcd for $C_{23}H_{33}O_8$ 437.2175).

**X-ray Crystallography of 1.** Compound **1** crystallized from MeOH as colorless needles. One needle (0.03 × 0.03 × 0.33 $mm^3$) was centered on the goniometer of an Oxford Diffraction SuperNova diffractometer operating with Cu K$\alpha$ radiation. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro.[26] The Laue symmetry and systematic absences were consistent with the monoclinic space groups *C*2, *Cm*, and *C*2/*m*. As the molecule was known to be enantiomerically pure, the chiral space group, *C*2, was chosen. The structure was solved using SHELXS-97[27] and refined using SHELXL-97[27] via OLEX2.[28] The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. The absolute configuration was established from anomalous dispersion effects (Flack *x* = 0.02(15);[29] Hooft P2(true) = 1.000, P3(true) = 1.000, P3(rac-twin) = 0.3 × $10^{-5}$; P3(false) = 0.7 × $10^{-25}$, *y* = 0.06(9)).[30,31]

*Crystal Data:* $C_{23}H_{32}O_8$, $M_r$ =436.49, monoclinic, *C*2, *a* = 32.7331(12) Å, *b* = 7.1315(3) Å, *c* = 9.7799(3) Å, $\alpha$ = 90.00°, $\beta$ = 92.961(3), $\gamma$ = 90.00, *V* = 2279.94(13) Å$^3$, 13 543 reflections, 287 parameters. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, are deposited at the Cambridge Crystallographic Data Centre (Deposition No. CCDC 802814).

*Athrolide B, (1S,2S,4R,5R,6R,7S,8S,10R)-2-(2-methylpropanoyloxy)-4-acetoxy-6-hydroxyguai-11(13)-en-8,12-olide (**2**):* white solid; $[\alpha]_D^{23}$ = −4° (*c* 0.2, $CHCl_3$); CD $[\theta]_{230}$ = −1560 (MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 211 nm (3.9); IR $\nu_{max}$ 3474, 2972, 1729, 1464, 1376, 1250, 1160, 1044, 1017 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) and $^{13}$C NMR (125 MHz, $CDCl_3$), see Table 1; HR-ESIMS *m/z* 417.1889 $[M + Na]^+$ (calcd for $C_{21}H_{30}NaO_7$ 417.1889).

*Athrolide C, 1S*,7R,10(H)R*-4-oxo-6S*-[(Z)-2-methyl-2-butenoyloxy]-8S*-(3-hydroxy-3-methylbutanoyloxy)pseudoguaia-2(3),11-(13)-dien-9R*,12-olide (**3**):* white solid; $[\alpha]_D^{23}$ = −69° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 220 nm (4.2); IR $\nu_{max}$ 3444, 2924, 1721, 1458, 1382, 1229, 1154, 1035, 999.8 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) and $^{13}$C NMR (125 MHz, $CDCl_3$), see Table 1; HR-ESIMS *m/z* 483.2003 $[M + Na]^+$ (calcd for $C_{25}H_{32}NaO$ 483.1995).

*Athrolide D, 1S*,7R*,10(H)R*-4-oxo-6S*-[(Z)-2-methyl-2-butenoyloxy]-8S*-(3-hydroxy-3-methylpentanoyloxy)pseudoguaia-2-(3),11(13)-dien-9R*,12-olide (**4**):* white solid; $[\alpha]_D^{23}$ = −66° (*c* 0.2, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 220 nm (4.3); IR $\nu_{max}$ 3445, 2927, 1721, 1458, 1382, 1216, 1154, 1035, 999.5 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) and $^{13}$C NMR (125 MHz, $CDCl_3$), see Table 1; HR-ESIMS *m/z* 497.2143 $[M + Na]^+$ (calcd for $C_{26}H_{34}NaO_8$ 497.2151) .

*Athrolide E, 1S*,7R*,10(H)R*-4-oxo-6S*-(E)-3-methyl-2-pentenoyloxy-8S*-(3-hydroxy-3-methylpentanoyloxy)pseudoguaia-2-(3),11(13)-dien-9R*,12-olide (**5**):* white solid; $[\alpha]_D^{23}$ = −33° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 221 nm (4.3); IR $\nu_{max}$ 3441, 2923, 1721, 1458, 1380, 1216, 1142, 1034, 1005.5 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) and $^{13}$C NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 489.2496 $[M + H]^+$ (calcd for $C_{27}H_{37}O_8$ 489.2488).

## ■ ASSOCIATED CONTENT

### Supporting Information

Figures giving $^1$H, $^{13}$C, COSY, HMBC, HMQC, and NOESY spectra of athrolides A–E (**1**–**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ ACKNOWLEDGMENTS

This project was supported by the Fogarty International Center, the National Cancer Institute, the National Science Foundation, the National Heart, Lung and Blood Institute, the National Institute of Mental Health, the Office of Dietary Supplements, and the Office of the Director of NIH, under Cooperative Agreement U01 TW000313 with the International Cooperative Biodiversity Groups. This project was also supported by the Agricultural Food Research Initiative of the National Institute of Food and Agriculture, USDA, Grant No. 2008-35621-04732, and by the National Science Foundation under Grant No. CHE-0619382 for purchase of the Bruker Avance 600 NMR spectrometer and Grant No. CHE-0722638 for the purchase of the Agilent 6220 mass spectrometer. This support is gratefully acknowledged. We thank Mr. B. Bebout and Dr. Mehdi Ashraf-Khorassani for obtaining the mass spectra and Dr. Hugo Azurmendi for assistance with the NMR spectra. We thank Dr. David Newman (National Cancer Institute) for the supply of the crude extract N110635.

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