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ANTIPLASMODIAL DITERPENOIDS FROM PSIADIA ARGUTA

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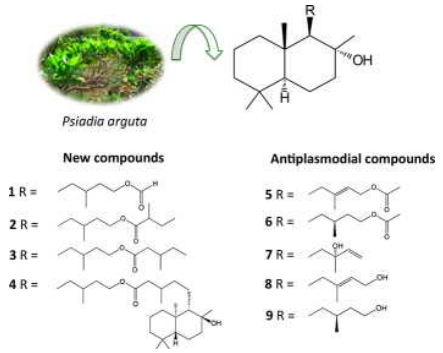
ABSTRACT: An ethyl acetate extract of *Psiadia arguta* leaves showed in vitro antiplasmodial activity against *Plasmodium falciparum* with IC_{50} values of 12.3 ± 2.4 μg/mL (3D7 strain) and 13.5 ± 3.4 μg/mL (W2 strain). Phytochemical investigation led to the isolation and characterization of 16 compounds including four new diterpenoids: labdan-8α-ol-15-yl-(formate) (**1**), labdan-8α-ol-15-yl-(2- methylbutanoate) (**2**), labdan-8α-ol-15-yl-(3-methylpentanoate) (**3**), and labdan- 8α-ol-15-yl-(labdanolate) (**4**). The latter compounds were characterized by spectroscopic methods (1D and 2D NMR, HRMS, and IR). The in vitro antiplasmodial activities of all compounds were evaluated. The known compounds labdan-13(E)-en-8α-ol-15-yl acetate (**5**), labdan-8α-ol-15-yl acetate (**6**), 13-epi-sclareol (**7**), labdan-13(E)-ene-8α,15-diol (**8**), and (8R,13S)-labdane- 8α,15-diol (**9**) exhibited antiplasmodial effects, with IC_{50} values of 29.1, 33.2, 35.0, 36.6, and 22.2 μM, respectively



The genus *Psiadia* (Asteraceae) consists of approximately 60 species mainly found in Madagascar and the Mascarene Islands (La Reunion, Mauritius, and Rodrigues).[1] *Psiadia arguta* (Pers.) Voigt, commonly known as "Baume de l'île Plate", is endemic to Mauritius and can be found on Ilot Gabriel, a small islet off Mauritius's north coast. This species is used traditionally for treatment of pulmonary infections, respiratory disorders, cough, and indigestion and is also employed to heal minor wounds and burns.[2,3] Previous pharmacological investigations of *P. arguta* extracts revealed several biological properties including antimicrobial,[4] anti-

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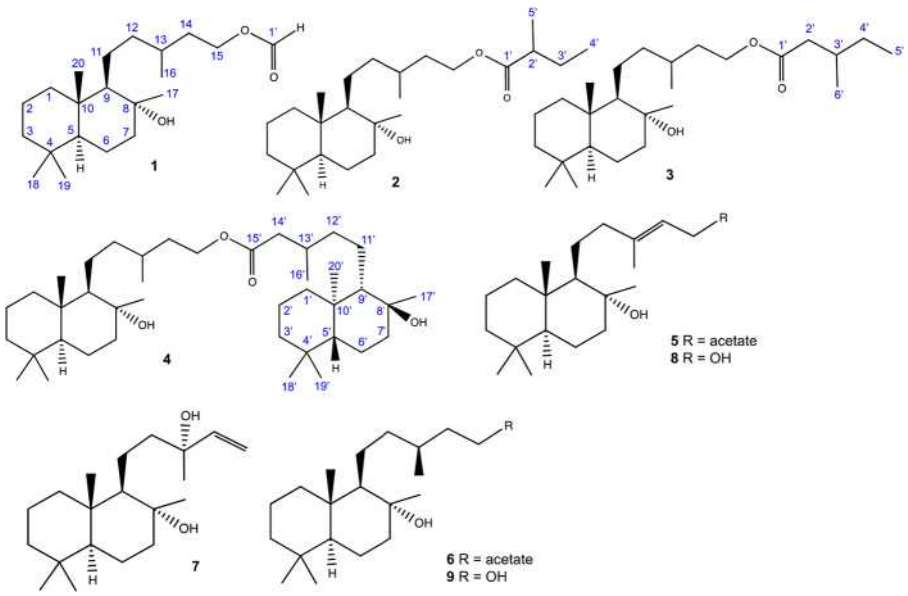
inflammatory,[5,6] antiplasmodial,[6] and cytotoxic[6] effects. Despite its biological potential, the phytochemistry of *P. arguta* has not previously been investigated, and thus this plant was selected for bioassay-guided fractionation and isolation of its antiplasmodial constituents. We herein report the isolation, structure elucidation, and in vitro antiplasmodial activity of four new (**1**-**4**) and 12 known diterpenoids from the EtOAc (ethyl acetate) extract of *P. arguta* leaves.

The *P. arguta* EtOAc leaf extract (yield 12%) was subjected to normal-phase chromatographic separation and yielded 18 fractions. The antiplasmodial activity of each fraction was evaluated. The promising antiplasmodial fractions F4, F6, F7, and F9 with IC_{50} values of 16.3, 8.8, 13.9, and 9.8 μg/mL, respectively, were further purified by semipreparative HPLC. The major compounds of the crude extract of *P. arguta* were found in fractions F11, F14, F15, and F18. These fractions were also subjected to purification of the major compounds. Column chromatography (CC) and repetitive reversed-phase preparative HPLC afforded the isolation of four new diterpenoids (**1**-**4**) and 12 known terpenoids. The latter were identified by comparison with published spectroscopic data as β-amyrin,[7] α-amyrin,[7] labdan-13(E)-en-8α-ol-15-yl acetate (**5**),[8] labdan-8α-ol-15-yl acetate (**6**),[9] anticopalic acid,[10] physanicandiol and 14-epi-physanicandiol,[11] *13-epi-* sclareol (**7**),[12] labdan-13(E)-ene-8α,15-diol (**8**),[13] (8R,13S)- labdane-8α,15-diol (**9**),[14] labdanolic acid,[15] and labdan-8(20)- en-15-ol.[16] To the best of our knowledge, this is the first report concerning the isolation of these compounds from *P. arguta*. The structures of the new compounds **1**-**4** were elucidated by the analysis of their 1D and 2D NMR and IR spectra and MS data and are described below.

Chart 1



Labdan-8α-ol-15-yl-(formate) (**1**) showed a $[M + Na]^+$ peak at *m/z* 361.2713 (calcd for $C_{21}H_{38}O_3Na$, 361.2713) in the HRESIMS, indicating a molecular formula of $C_{21}H_{38}O_3$ and suggesting the occurrence of three degrees of unsaturation. The IR spectrum exhibited a broad band at 3436 cm^{-1}, characteristic of a hydroxy group, and a band at 1728 cm^{-1}, characteristic of the carbonyl of the formate ester function. Comparison of the spectroscopic data (HRMS, NMR, IR) of **1** to those of the diterpenoid labdan-8α-ol-15-yl acetate[9] (**6**) ($C_{22}H_{40}O_3$) indicated substantial similarities between these two compounds, suggesting that compound **1** has one methyl group less than labdan-8α-ol-15-yl acetate (**6**).

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The ^{1}H, ^{13}C, and HMBC NMR spectra revealed resonances and $^{2,3}J_{HC}$ correlations consistent with those of an acetate moiety (δC 171.08 for C-1′ and δ_H 2.00 for H-2′) attached to C-15 in compound **6**, whereas in compound **1**, the signals of C-1′ (δC 161.4) linked to one proton H-1′ (δ_H 8.05, singlet) supported a formate moiety also attached to C-15 (Table 1). This information was confirmed by the HMBC cross-peak between H-15 (δ_H 4.25 and 4.18) and C-1′ (δC 161.4) (Figure 1). Therefore, **1** was assigned as labdan-8α-ol-15-yl-(formate).

The molecular formula of labdan-8α-ol-15-yl-(2-methylbu- tanoate) (**2**) was determined to be $C_{25}H_{46}O_3$ by HRESIMS analysis, which showed a $[M + Na]^+$ peak at *m/z* 417.3337 (calcd for $C_{25}H_{46}O_3Na$, 417.3339). This suggested the presence of three degrees of unsaturation. The IR spectrum exhibited a broad band at 3504 cm^{-1} that was indicative of the presence of a hydroxy group and two bands at 1734 and 1184 cm^{-1}, characteristic of an ester function. Comparison of the spectroscopic data (HRMS, NMR, IR) of **2** to those of labdan- 8α-ol-15-yl-(formate) (**1**) ($C_{21}H_{28}O_3$) indicated substantial similarities between these two compounds, suggesting that compound **2** has one butyl group more than labdan-8α-ol-15- yl-(formate) (**1**). Inspection of ^{1}H and ^{13}C NMR data (Table 1) suggested that C-2′ is a methine group (δC 41.3 and δ_H 2.35), C-3′ is a methylene (δC 26.9 and δ_H 1.67 and 1.44), and C-4′ (δ_c 11.8 and δ_H 0.90) and C-5′ (δ_c 16.8 and δ_H 1.13) are two methyl groups. With the aid of COSY and HMBC experiments, a 2-methylbutanoate moiety was identified by further analysis of the remaining ^{1}H NMR resonances [δ_H 2.35 (1H, sext, J = 7.0 Hz, H-2′), 1.67 (1H, ov, H-3′a), 1.44 (1H, ov, H-3′b), 1.13 (3H, d, *J* = 7.4 Hz, H-5′), and 0.90 (3H, t, *J* = 7.4 Hz, H-4′)] and by cross-peaks between H-2′ (δ_H 2.35), H- 3′ (δ_H 1.67 and 1.44), H-5′ (δ_H 1.13), and C-1′ (δC 177.1) (Figure 1). This 2-methylbutanoate moiety was assigned at the C-15 position, based on the HMBC cross-peak between H-15 (δ_H 4.15 and 4.06) and C-1′ (δC 177.1) (Figure 1).

Labdan-8α-ol-15-yl-(3-methylpentanoate) (**3**) exhibited a molecular formula of $C_{26}H_{48}O_3$, as determined by its ^{13}C NMR and HRESIMS data (molecular ion at *m/z* 431.3493 $[M + Na]^+$; calcd for $C_{26}H_{48}O_3Na$ 431.3496). Comparison of the spectroscopic data (HRMS, NMR, IR) of **3** with those of **2** ($C_{25}H_{46}O_3$) indicated that both compounds have almost the same skeleton and revealed that compound **3** has an amyl group at the C-15 position instead of a butyl group as in compound **2**. Analysis of the ^{1}H and ^{13}C NMR data indicated that this amyl chain contains one methine C-3′ (δ_c 32.1 and δ_H 1.87), two methylenes C-2′ (δC 41.8 and δ_H 2.09 and 2.29) and C-4′ (δC 29.5 and δ_H 1.22 and 1.36), and two methyls C5′ (δC 11.4 and δ_H 0.89) and C-6′ (δC 19.4 and δ_H 0.92). The amyl group could be assigned to 3-methylpentanoate by analysis of 2D NMR spectroscopic data including COSY and HMBC experiments.

Labdan-8α-ol-15-yl-(labdanolate) (**4**) showed a $[M + Na]^+$ peak at *m/z* 639.5328 (calcd for $C_{40}H_{72}O_4Na$, 639.5323) in the HRESIMS, indicating a molecular formula of $C_{40}H_{72}O_4$ and suggesting the occurrence of five degrees of unsaturation. Its IR spectrum exhibited similarities (bands at 3447, 1717, and 1183 cm^{-1}) to those of **2** and **3**, suggesting the presence of hydroxy groups and ester functions. The structure elucidation of **4** was established by comparison of its NMR data to those of **3**, with both exhibiting substantial similarities. The main difference was found for the signal of the methyl C-5′ (δ_c 11.4 and δ_H 0.89) in compound **3**, which corresponded to the methylene C-11′ (δ_c 20.3 and δ_H 1.28 and 1.37) in the case of compound **4**. Thus, this information suggested that compound **4** has a substituent at the C-11′ position in accordance with a molecular mass of 209.1905 (corresponding to a molecular formula of $C_{14}H_{25}O$). Moreover, duplicated signals of all carbons of the bicyclic labdane skeleton were apparent in the ^{13}C NMR spectrum, establishing the substitution at the C-11′ position as an identical bicyclic ring. The attachment of the latter at the C-11′ position was confirmed by a COSY correlation between H-11′ (δ_H 1.37 and 1.28) and H-9′ (δ_H 1.00) and HMBC correlations between H-12′ (δ_H 1.45 and 1.28) and H-9′ (δ_H 1.00) and C-11′ (δ_C20.3) (Figure 1).

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***Table 1**. [1]H (600 MHz) and [13]C (150 MHz) NMR Data for Compounds 1-4 ($CDCl_3$ at 300 K)*

	1		2		3		4	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H,mult (*J* in Hz)
1	39.9, CH_2	1.61, ov;[a] 0.91, td (13.1, 3.4)	39.9, CH2	1.60, ov; 0.93, ov	39.9, CH2	1.61, ov; 0.93, td (13.1, 3.4)	39.8, CH2	1.61, ov; 0.93, ov
2	18.6, CH_2	1.59, ov; 1.42, ov	18.6, CH2	1.58, ov; 1.42, ov	18.6, CH2	1.58, ov; 1.42, ov	18.6, CH2	1.59, ov; 1.42, ov
3	42.2, CH_2	1.38, dm (13.0); 1.14, td (13.6, 4.1)	42.2, CH_2	1.37, dm (13.0); 1.13, ov	42.2, CH_2	1.37, dm (13.0); 1.13, ov	42.2, CH_2	1.38, ov; 1.14, br t (13.6)
4	33.4, CH_2		33.4, CH_2		33.4, CH_2		33.4, CH_2	
5	56.3, CH	0.91, dd (12.4, 2.4)	56.3, CH	0.90, ov	56.3, CH	0.91, br d (12.6)	56.28, CH	0.91, ov
6	20.7, CH_2	1.65, ov; 1.26, ov	20.7, CH_2	1.64, ov; 1.26, ov	20.7, CH_2	1.64, ov; 1.26, ov	20.68, CH_2	1.65, ov; 1.26, ov
7	44.7, CH_2	1.86, dt (12.3, 3.2); 1.39, ov	44.6, CH_2	1.86, dt (12.4, 3.2); 1.38, ov	44.6, CH_2	1.86, dt (12.3, 3.2); 1.38, ov	44.5, CH_2	1.86, dt (12.4, 3.4); 1.39, ov
8	74.5, C		74.6, C		74.4, C		74.39, C	
9	62.6, CH	1.00, br t (3.9)	62.6, CH	1.00, br t (3.8)	62.6, CH	1.00, br t (3.9)	62.5, CH	1.00, br t (3.9)
10	39.3, C		39.3, C		39.3, C		39.29, C	
11	23.0, CH_2	1.32, ov	23.1, CH2	1.31, ov	23.1, CH2	1.31, ov	23.0, CH_2	1.37, ov; 1.28, ov
12	41.2, CH_2	1.46, ov; 1.23, ov	41.2, CH_2	1.46, ov; 1.22, ov	41.2, CH_2	1.46, ov; 1.22, ov	41.2, CH_2	1.46, ov; 1.20, ov
13	30.9, CH	1.53, ov	30.9, CH	1.51, ov	31.0, CH	1.51, ov	30.9, CH	1.51, ov
14	35.6, CH_2	1.70, ov; 1.48, ov	35.8, CH2	1.66, ov; 1.43, ov	35.8, CH2	1.66, ov; 1.44, ov	35.8, CH2	1.66, ov; 1.43, ov
15	62.6, CH_2	4.25, ddd (11.0, 7.6, 6.0); 4.18 dt, (11.0, 7.0)	62.8, CH_2	4.15, ddd (10.9, 7.5, 6.0); 4.06 dt, (10.9, 7.1)	62.8, CH_2	4.14, ddd (11.0, 7.7, 6.0); 4.06 dt, (11.0, 7.0)	62.6, CH_2	4.14, ddd (10.9, 7.5, 6.0); 4.06, dt (10.9, 7.1)
16	19.6, CH_3	0.93, d (6.5)	19.6, CH_3	0.91, d (6.5)	19.6, CH_3	0.92, d (6.5)	19.6, CH_3	0.92, d (6.5)
17	24.1, CH_3	1.14 s	24.1, CH3	1.13 s	24.1, CH3	1.14 s	24.1, CH3	1.14 s
18	33.6, CH3	0.86 s	33.6, CH3	0.86 s	33.6, CH3	0.87 s	33.6, CH3	0.86 s
19	21.6, CH_3	0.79 s	21.6, CH_3	0.78 s	21.7, CH_3	0.79 s	21.7, CH_3	0.78 s
20	15.7, CH3	0.79 s	15.6, CH3	0.79 s	15.6, CH3	0.79 s	15.6, CH3	0.79 s
1′	161.4, C	8.05 s	177.1, C		173.7, C		39.9, CH2	1.61, ov; 0.93, ov
2′			41.3, CH	2.35, sext (7.0)	41.8, CH2	2.29, dd (14.6, 6.2); 2.09, dd (14.6, 8.4)	18.6, CH_2	1.59, ov; 1.42, ov
3′			26.9, CH2	1.67, ov; 1.44, ov	32.1, CH	1.87, ov	42.2, CH2	1.38, ov; 1.14, br t (13.6)
4′			11.8, CH3	0.90, t (7.4)	29.5, CH2	1.36, ov; 1.22, ov	33.5, CH2	
5′			16.8, CH_3	1.13, d (7.4)	11.4, CH_3	0.89, t (7.3)	56.30, CH	0.91, ov
6′					19.4, CH_3	0.92, d (6.8)	20.72, CH_2	1.65, ov; 1.26, ov
7′							44.7, CH2	1.86, dt (12.4, 3.4), 1.39, ov
8′							74.42, C	
9′							62.7, CH	1.00, br t (3.9)
10′							39.32, C	
11′							20.3, CH_2	1.37, ov; 1.28, ov
12′							29.8, CH2	1.45, ov; 1.28, ov

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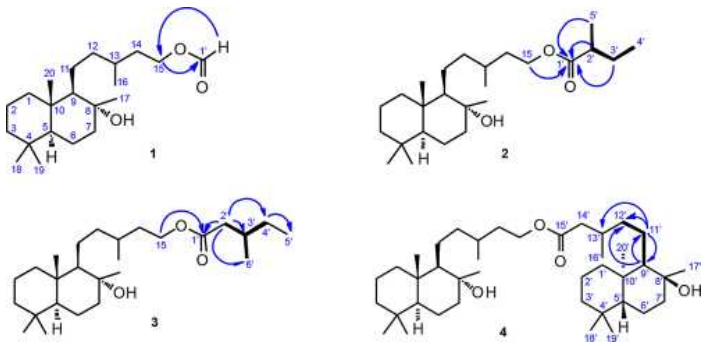
13′							31.5, CH	1.92, oct (6.8)
14′							41.9, CH2	2.30, dd (14.6, 5.9); 2.14, dd (14.6, 7.9)
15′							173.7, C	
16′							20.1, CH3	0.92, d (6.7)
17							24.2, CH_3	1.14 s
18′							33.6, CH3	0.86 s
19′							21.7, CH_3	0.78 s
20′							15.7, CH3	0.79 s

[a]*ov : overlapped.*

The absolute configurations of the new compounds were not elucidated due to the paucity of each sample. However, the relative configuration could be assigned using the method of Carman,[17] which established that the optical rotation value should give information about the structure and stereochemistry of the molecule.

Figure 1. *Key COSY (bold) and HMBC (blue arrows) correlations for compounds* ***1***-***4****.*



This study on the empirical optical rotation values of labdane derivatives suggested that the optical rotation value should be the addition of the decalin ring portion and the lateral chain portion. However, application ofthis method did not allow a determination of the relative configurations of all new compounds, particularly for compound **1**, where the lack of chiral center in the formate substituent did not permit the application of this procedure. Therefore, the relative configuration of these four new compounds has been established based on their NOESY spectra. Regarding the bicyclic ring, the relative configurations at C-5, C-8, C-9, and C-10 have to be defined. In their NOESY spectra, compounds **1**-**4** displayed the same NOE correlations for the decalin ring. The NOE cross-peaks H-20/H-17, H-20/ H-11, and H-5/H-9 and the lack of NOE correlations between H-20, H-17, H-11 and H-5, H-9 (Figure 2) suggested that the bicyclic ring configuration has to be *normal-* or *ent-.* The NOE correlations observed for these four new compounds were compared to those of the known isolated analogues: the NOE correlations were similar for all diterpenes isolated.

As all the labdanes isolated were in the *normal-* configuration, it was considered that the configuration of decalin ring of all the new compounds is the same. Confirmation of the configuration in the decalin ring in each case was determined by comparison of observed correlations and chemical shifts of protons and carbons near the chiral centers with those of the known compounds: 13-*epi*-sclareol (**7**)[12] and labdanolic acid.[15] This information established the decalin ring as *normal-* configuration. However, configurations of chiral centers C-13, C-2′, C-3′, and C-13′ located on the lateral chains have not been established.

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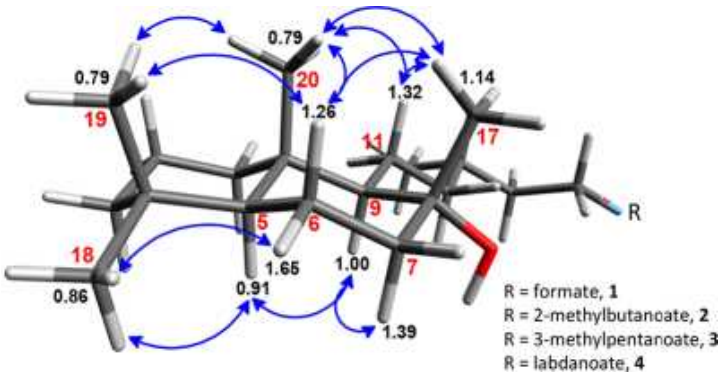
Figure 2. *Key NOESY (blue arrows) correlations observed for the decalin nucleus of compounds* ***1***-***4****.*



All the isolated compounds were tested for antiplasmodial activity against the 3D7 strain of *Plasmodium falciparum* (Table 2). Five of the known compounds exhibited moderate antiplasmodial activity: labdan-13(*E*)-en-8α-ol-15-yl acetate (**5**), labdan-8α-ol-15-yl acetate (**6**), 13-*epi*-sclareol (**7**), labdan- 13(E)-ene-8α,15-diol (**8**), and (8R,13S)-labdane-8α,15-diol (**9**), with IC_{50} values of 29.1, 33.2, 35.0, 36.6, and 22.2 μM, respectively.

Table 2. *Antiplasmodial and Cytotoxic Activities of the Isolated Compounds 1-9*

compound	*P. falciparum* (3D7) IC_{50} (μM)[a]
labdan-8α-ol-15-yl-(formate) (**1**)	73.0
labdan-8α-ol-15-yl-(2-methylbutanoate) (**2**)	58.0
labdan-8α-ol-15-yl-(3-methylpentanoate) (**3**)	54.8
labdan-8α-ol-15-yl-(labdanolate) (**4**)	inactive[b]
labdan-13(E)-en-8α-ol-15-yl acetate (**5**)	29.1
labdan-8α-ol-15-yl acetate (**6**)	33.2
13-epi-sclareol (**7**)	35.0
labdan-13(E)-ene-8α,15-diol (**8**)	36.6
(8R,13S)-labdane-8α,15-diol (**9**)	22.2
artemisinin	0.010

[a]Values are the means ± standard deviations calculated from three independent assays in duplicate for antiplasmodial activity and two independent assays in triplicate for cytotoxicity activity. [b]Inactive: IC_{50} > 50 μg/mL.

None of the compounds isolated is likely to be an antimalarial drug candidate because their IC_{50} values are in the μM range. However, these compounds could be used as starting materials for the semisynthesis of labdane derivatives with better antiplasmodial properties. Indeed, studies have suggested the relevance of a lactone or an endoperoxide group (such as artemisinin and its derivatives) to interfere with the parasite development.[18,19] The semisynthesis of labdane derivatives from manool and sclareol has been previously reported.[19-21]

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EXPERIMENTAL SECTION

GENERAL EXPERIMENTAL PROCEDURES

Optical rotations were recorded on an Anton Paar MCP200 polarimeter. IR spectroscopic data were measured on a FTIR Bruker Tensor 27 spectrometer. NMR spectra were recorded in $CDCl_3$ on a Bruker NMR spectrometer operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. The chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz. The spectra were processed using 1D and 2D MestReNova (Mnova 12.0 Mestrelab Research) software. HRESIMS were obtained on a SYNAPT G2 HDMS (Waters) TOF mass spectrometer in the positive-ion mode.

Column chromatography was carried out using a glass column packed with Merck silica gel (0.040-0.063 mm; 230-400 mesh). Semipreparative HPLC was carried out using Phenomenex Gemini C_{18} Prep (250 × 10 mm; i.d. 5 μm) or Phenomenex Synergi hydro C_{18} Prep (250 × 10 mm; i.d. 5 μm) columns and was performed on Dionex Ultimate 3000 (Thermo Scientific) equipped with photodiode array detector (Thermo Scientific). Analytical HPLC was carried out using Gemini C_{18} (150 × 4.6 mm; i.d. 5 μm) or Synergi hydro C_{18} (150 × 4.6 mm; i.d. 5 μm) columns. All solvents were analytical or HPLC grade.

PLANT MATERIAL

Psiadia arguta leaves were collected in the dry season in January 2015 in Mauritius (Réduit). A voucher specimen was deposited in the Herbarium of The University of Mauritius for identification, and the accession number MAU0018969 was attributed to the sample. Leaves were dried at 40 °C and kept at room temperature until processed.

EXTRACTION AND ISOLATION

Dried and powdered leaves of *P. arguta* (110 g) were extracted exhaustively using an accelerated solvent extractor, ASE 300. Four successive extractions were carried out at 40 °C with EtOAc. The extract was evaporated under reduced pressure to yield about 12 g of crude extract. Nine grams of the crude extract was fractionated by column chromatography on normal-phase silica gel 60 (35 × 5 cm) using combinations of *n*-hexane, EtOAc, and MeOH of increasing polarity. Altogether, 18 fractions were obtained. All fractions were evaluated for their antiplasmodial activity against *P. falciparum.* The active fractions found were fractions F4, F6, F7, and F9 (IC_{50} = 16.3, 8.8, 13.9, and 9.8 μg/mL, respectively), and fractions containing major compounds were fractions F11, F14, F15, and F18. Fraction 4 was subjected to semipreparative HPLC (Synergi hydro 5 μm, 250 × 10 mm i.d.), 4.5 mL min^{-1} isocratic elution with 95% MeOH-H_2O (+0.1% formic acid) over 2 min, followed by 97% MeOH-H_2O over 2 min, then 99% MeOH-H_2O over 1 min and maintained at 100% MeOH over 20 min (UV 210 nm). The purification of fraction 4 led to isolation of pure compounds labd- 8(20)-en-15-ol (1.3 mg, t_R 16.5 min) and α-amyrin (2.6 mg, t_R 15 min). Fraction 4 was further purified by CC on silica gel (30 × 2.5 cm), using a gradient of n-hexane-EtOAc (100:0 to 0:100, v/v) as mobile phase and gave 14 fractions. Subfraction F4-f2 was subjected to semipreparative HPLC (Gemini C_{18} prep column, 5 μm, 250 × 10 mm i.d.), 5 mL min^{-1} isocratic elution with 95% MeOH-H_2O (+0.1% formic acid) over 2 min and elution gradient to 100% MeOH (+0.1% formic acid) over 10 min and maintained at 100% MeOH over 18 min; UV 210 nm. Pure compounds β-amyrin (3.7 mg, t_R 18.6 min) and α-amyrin (8.2 mg, t_R 19.8 min) were thus obtained. The purification of subfraction F4-f6 was performed by semipreparative HPLC (Gemini C**1**$_8$ prep column, 5 μm, 250 × 10 mm i.d.), 5 mL min^{-1} gradient elution with 90% MeOH--H_2O (+0.1% formic acid) to 100% over 10 min and maintained at 100% MeOH over 25 min (UV 210 nm). Purification yielded pure compound **3** (6.2 mg, t_R 12.5 min).

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Fraction 6 was subjected to semipreparative HPLC (Gemini C_{18} prep column, 5 μm, 250 × 10 mm i.d.), 5 mL min^{-1} gradient elution with 85% MeOH-H_2O (+0.1% formic acid) to 92.5% MeOH-H_2O (+0.1% formic acid) over 15 min (UV 210 nm), and furnished pure compounds **5** (3.7 mg, t_R 11.5 min), **6** (5.7 mg, t_R12.9 min), and **1** (1.4 mg, t_R12.3 min).

Purification of fraction 7 was performed by semipreparative HPLC (Gemini C_{18} prep column, 5 μm, 250 × 10 mm i.d.), 4 mL min^{-1} isocratic elution with 92% MeOH-H_2O (+0.1% formic acid) over 5 min and gradient elution with 92% MeOH-H_2O (+0.1% formic acid) to 100% MeOH-H_2O (+0.1% formic acid) over 10 min (UV 210 nm). Fraction 7 afforded pure compound anticopalic acid (1.7 mg, t_R 9.9 min). Fraction 9 was applied to semipreparative HPLC (Gemini C_{18} prep column, 5 μm, 250 × 10 mm i.d.), 5.5 mL min^{-1}, and eluted by a 85% to 100% MeOH-H_2O (+0.1% formic acid) gradient over 30 min to yield physanicandiol and 14-epi-physanicandiol (mixture 50:50, 1.7 mg, t_R 9.5 min) and **4** (1.8 mg, t_R 25.5 min). Fraction 11 was submitted to CC on silica gel (18 × 1.5 cm, 230-400 mesh), using a gradient of n- hexane-EtOAc (100:0 to 40:60) as mobile phase. The purification afforded compound **7** (6 mg). Fraction 14 was purified by CC on silica gel (20 × 1.5 cm, 230-400 mesh), using a gradient of *n*- hexane-EtOAc (75:25 to 50:50) as mobile phase to give pure compound **8** (1.65 mg). Fraction 15 yielded compound **9** (0.5 g) without further purification. Fraction 18 was purified by CC on silica gel (12 × 3 cm, 230-400 mesh), using a gradient of n-hexane-EtOAc (50:50 to 10:90) as mobile phase to give pure labdanolic acid (9 mg).

Labdan-8α-ol-15-yl-(formate) (1): green oil; [α]$\mathbf{D}^5$ -78 (c 0.11, $CHCl_3$); IR (ATR) ν_{max} 3436, 2928, 2869, 1728, 1460, 1387, 1365, 1173 cm^{-1}; ^1H and ^{13}C NMRdata, Table 1; HRESIMS *m/z* 361.2713 $[M + Na]^+$ (calcd for $C_{21}H_{38}O_3Na$, 361.2713).

Labdan-8α-ol-15-yl-(2-methylbutanoate) (2): colorless oil; [α]$\mathbf{D}^5$ -9 (c 1.17, $CHCl_3$); IR (ATR) ν_{max} 3504, 2927, 2869, 2855, 1734, 1719, 1461, 1386, 1365, 1261, 1184, 756 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; $C_{25}H_{46}O_3$; HRESIMS *m/z* 417.3337 $[M + Na]^+$ (calcd for C**25**H**46**O**3**Na, 417.3339).

Labdan-8α-ol-15-yl-(3-methylpentanoate) (3): colorless oil; [α]$\mathbf{D}^5$ -6 (c 0.64, $CHCl_3$); IR (ATR) ν_{max} 3505, 2959, 2929, 2872, 1721, 1461, 1387, 1365, 1260, 1183, 755 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; $C_{26}H_{48}O_3$; HRESIMS *m/z* 431.3493 $[M + Na]^+$ (calcd for $C_{26}H_{48}O_3Na$, 431.3496).

Labdan-8α-ol-15-yl-(labdanolate) (4): green gum; [α]$\mathbf{D}^5$ +6 (*c* 0.34, $CHCl_3$); IR (ATR) ν_{max} 3447, 2927, 2854, 1717, 1459, 1388, 1260, 1183, 755 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS m/ z 639.5328 $[M + Na]^+$ (calcd for $C_{40}H_{72}O_4Na$, 639.5323).

IN VITRO ANTIPLASMODIAL ASSAYS

The effect of each fraction and pure compound on parasite growth of the P. *falciparum* strain 3D7 was measured in a 48 h growth assay as previously reported.[22,23] Briefly, ring-stage parasite cultures were grown for 48 h in the presence of increasing concentration of drug at 37 °C. After 48 h in culture, parasite viability was determined by the APAD colorimetric test. The half inhibitory concentration (IC_{50}) values are the average of three independent assays with each determination in duplicate and are expressed as means ± SEM.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00698.

HRESIMS, IR (FT-IR), and 1D and 2D NMR spectra for **1**-**4** (PDF)

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The authors declare no competing financial interest.

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