

Antiplasmodial Metabolites Isolated from the Marine Octocoral *Muricea austera*

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Bioassay-guided fractionation of the MeOH extract from the octocoral *Muricea austera* collected in the Pacific coast of Panama led to the isolation of eight compounds, including three tyramine derivatives (**1–3**), two steroidal pregnane glycosides (**4, 5**), and three sesquiterpenoids (**6–8**). Compounds **2–5** are new natural products, and their structures were determined on the basis of their spectroscopic data (HRMS, 1D and 2D NMR, and CD studies). The antiprotozoal activities of the natural compounds **1–8** as well as those of a series of synthetic glycosides (**11–22**) and tyramine derivatives (**23–35**) were evaluated in vitro against a drug-resistant *Plasmodium falciparum* and intracellular form of *Trypanosoma cruzi*.

Marine organisms are an extremely rich source of novel and bioactive natural products.¹ Among them, gorgonian octocorals constitute one of the more interesting groups due to their ability to synthesize a variety of compounds with chemical structures that have no equivalent in the terrestrial environment.² Recent studies carried out at Coiba National Park (CNP) in the Republic of Panama revealed that this region possesses a great diversity of octocoral species.³ Although the natural products chemistry of marine invertebrates in CNP remains largely unexplored, some efforts are being directed to increase the knowledge of the chemistry and biology of these organisms.^{4–6} As a part of these efforts, we are exploring the chemistry of some sponges and gorgonian octocorals collected from CNP using a variety of biological assays for drug discovery against cancer, malaria, leishmaniasis, and Chagas' disease. In this article we communicate the isolation and the antiprotozoal activity of compounds **1–8** isolated from the octocoral *Muricea austera* (Verrill, 1869; Plexauridae) collected from CNP. Compounds **2–5** are new natural products, and their structures were established on the basis of their HRMS, NMR spectroscopic data, and CD studies. Preliminary studies related to the antiprotozoal activity of the active natural products were carried out using a series of glycosides and tyramine derivatives obtained through synthesis.

Results and Discussion

Specimens of *M. austera* were collected from CNP during an expedition of the Smithsonian Tropical Research Institute. The MeOH crude extract of *M. austera* showed in vitro activity against chloroquine-resistant *P. falciparum* and was subjected to activity-guided fractionation using solvent partition and vacuum liquid chromatography (VLC), followed by silica gel flash chromatography and normal-phase HPLC purification to yield compounds **1–8**. The structures of the new metabolites **2–5** were deduced using HRESI-TOF MS, NMR (¹H, ¹³C, DEPT, COSY, HSQC, HMBC, NOE, and ¹H decoupling) analysis, and CD studies. Compounds **1**⁷ and **6–8**⁸ were identified by comparison of their spectroscopic data with those described in the literature.

The optically active compound **2** ($[\alpha]_D^{25} -26.8$) analyzed for C₂₁H₃₅NO₂ by HRESI-TOFMS and ¹³C NMR. Five degrees of

unsaturation were calculated from the molecular formula. NMR spectroscopic data of compound **2** allowed the construction of two substructures. In the first, an intense broad singlet at δ_H 1.25 ppm (14 H) and two methyl groups at δ_H 0.87 (3H) and δ_H 0.88 (3H) clearly suggested the presence of a fatty acid moiety. On the other hand, the AA'XX' system formed by two doublets at 7.03 (2H, d, $J = 8.4$ Hz) and 6.78 ppm (2H, d, $J = 8.4$ Hz), indicative of a *p*-disubstituted aromatic ring and the presence of a pair of methylenes mutually coupled at δ_H 2.73 (H-7', t, $J = 6.8$ Hz) and 3.48 (H-8', dd, $J = 6.8, 12.0$ Hz), together with a deuterium interchangeable amide proton at δ_H 5.44, revealed a tyramine unit. Both substructures were confirmed by fragments at m/z 120 (100%) and 214 (78%) observed in the EIMS spectra.

The five degrees of unsaturation were accounted for by the aromatic ring of the tyramine moiety and the amide carbonyl. Therefore, the fatty acid residue must be formed by a saturated C₁₂ carbon chain. The carbon at δ_C 14.1 (C-12) showed the characteristic chemical shift for a terminal CH₃ in a fatty acid chain, while the remaining methyl group at δ_C 19.6 (C-13) was attached to C-3 on the basis of COSY experiments and its ^{2,3}*J* HMBC correlations with carbons C-2, C-3, and C-4 (Figure 1a). The diastereotopic character of the methylene protons at δ_H 2.12 and 1.86 (H-2a and H-2b) indicated their proximity to a chiral center, and their position was defined by their ^{2,3}*J* HMBC correlations with carbons C-1 and C-13 (Figure 1a). Thus, compound **2** was identified as *N*-[2-(4-hydroxyphenyl)ethyl]-3-methyldodecanamide.

Compound **3** was consistent with an empirical formula of C₂₂H₃₁NO₂ by HRESI-TOFMS and ¹³C NMR and consistent with 8 degrees of unsaturation. ¹H and ¹³C NMR spectroscopic data of compound **3** had some similarities with those of **2**, indicating that **3** was also a tyramine fatty acid derivative. Five degrees of unsaturation can be accounted for by the tyramine moiety and the amide carbonyl. The remaining unsaturations were accounted for by three carbon–carbon double bonds formed by six olefinic carbon atoms at 126.9, 127.9, 128.5, 128.8, 128.9, and 132.1 ppm located on the fatty acid chain. The spin systems formed by H-2, H-3, H-4, H-5 and H-12, H-13, H-14 were assigned through COSY experiments and their ^{2,3}*J* HMBC correlations (Figure 1b). The *cis*-geometries of the three double bonds of the polyunsaturated C₁₄ fatty acid residue were assigned by ¹³C NMR, since the resonances observed for the olefinic carbons (C-5, C-6, C-8, C-9, C-11, and C-12) as well as those of the allylic methylenes (C-7 and C-10) showed the typical δ_C values for a methylene-interrupted *cis* double-bond system.^{9–11} The positions of the remaining carbons were

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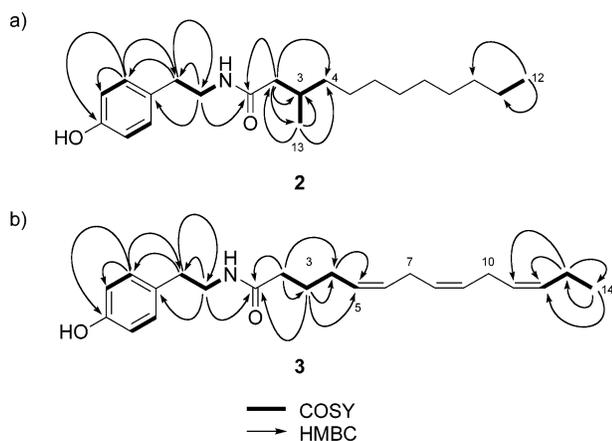


Figure 1. Selected 2D NMR correlations of compounds **2** (a) and **3** (b).

defined by comparison of their experimental δ_C with those calculated theoretically following the method described by Sandri et al.⁹

Compound **4** analyzed for $C_{28}H_{42}O_6$ by HRESI-TOFMS and ^{13}C NMR. DEPT and HSQC experiments revealed four quaternary carbons, 11 methines, 10 methylenes, and three methyl groups. Signals observed for an acetal carbon at 97.5 ppm joined by three oxygen-bonded methines and one methylene at 73.0, 68.3, 67.2, and 62.4 ppm, respectively, suggested the presence of a pentose sugar unit in the molecule. A deshielded methyl group at δ_H 2.18 and a carbonyl carbon at δ_C 170.8 corresponded to an acetate group that was attached to C-3' of the sugar unit on the basis of the 3J HMBC correlation observed between one of the sugar methines (H-3', 5.09 ppm) and the acetate carbonyl. An analysis of the 1H NMR coupling constants in addition to the data provided by COSY, HMBC, and proton decoupling experiments allowed the sugar to be identified as 3'-*O*-acetyl- β -D-arabinopyranose. The remaining 21 carbon atoms and the intense EIMS peak observed at m/z 282 (100%) matched with the C_{21} pregnane steroidal aglycon,¹² which has also been identified in other *Muricea* species.¹³ The attachment of the sugar moiety to C-3 of the aglycon was deduced from the 3J HMBC correlation between the anomeric proton (H-1', 5.06 ppm) and C-3 (77.8 ppm). Overall, the data provided by COSY, HSQC, and HMBC correlations and MS allowed the assignment of the structure of compound **4** as 3'-*O*-acetyl- β -pregna-5,20-dienyl- β -D-arabinopyranoside.

Compound **5** was consistent with $C_{28}H_{42}O_6$ by HRESI-TOFMS and ^{13}C NMR. This compound displayed the same molecular formula as **4**, similar NMR spectroscopic features, and the same fragmentation pattern in the EIMS. These similarities suggested that **4** and **5** were isomers. After a detailed examination of 1D and 2D NMR spectroscopic data of **5**, we observed that the only difference between these compounds was the position of the acetate group on the arabinose unit. Through the analysis of 1H NMR coupling constants, COSY, HMBC, and proton decoupling experiments, we identified the sugar in **5** as 4'-*O*-acetyl- β -D-arabinopyranose. Thus, compound **5** was identified as 4'-*O*-acetyl- β -pregna-5,20-dienyl- β -D-arabinopyranoside.

A base hydrolysis of the acetate groups in **4** and **5** was carried out separately using LiOH in THF- H_2O . In both cases, compound **9** was obtained, confirming the isomeric nature of **4** and **5**. In order to determine the absolute configuration of the sugar in **4** and **5**, we carried out circular dichroism (CD) studies on derivative **10** (obtained by perbenzoylation of compound **9** with benzoyl chloride in pyridine) and the synthetic standard **12** (prepared from commercial L-arabinopyranose). The CD spectra of glycoside **10** showed a Davydov splitting identical with the standard **12** but with the opposite chirality, indicating that the sugars were enantiomeric.

Table 1. Activity of Compounds **1–22** against Chloroquine-Resistant *Plasmodium falciparum* and Intracellular *Trypanosoma cruzi*^a

compd	<i>P. falciparum</i>		<i>P. falciparum</i>		compd	<i>T. cruzi</i>	
	IC ₅₀ (μ M)						
1	36	>50	12	35	>50		
2	45	>50	13	21	>50		
3	38	ND	14	>50	>50		
4	67	96	15	>50	>50		
5	80	101	16	>50	>50		
6	42	173	17	>50	>50		
7	ND	ND	18	>50	>50		
8	>50	ND	19	43	>50		
9	ND	ND	20	36	>50		
10	ND	ND	21	29	>50		
11	28	28	22	>50	>50		
chloroquine	0.07						
nifurtimox		11					

^a ND = not determined

Given that the absolute configuration of the synthetic standard (**12**) was "L", we assigned the absolute configuration of the sugar in **10**, and therefore in the natural products **4** and **5**, as "D".

The hexane fraction obtained by solvent partition of the crude extract showed antiplasmodial activity and was subjected to VLC silica gel chromatography and normal-phase HPLC purification to yield the germacrenes **6** and **7** and the elemene sesquiterpene **8**; these were identified by comparison of their spectroscopic data with those described in the literature.⁸

The antiplasmodial activities of the natural products **1–8** were evaluated in vitro against chloroquine-resistant *P. falciparum*.¹⁴ Compounds **1–6** showed moderate antiplasmodial activity, with IC₅₀ values between 11 and 38 μ g/mL, while compound **8** showed no measurable activity (Table 1). The antiplasmodial activity of the glycosides **4** and **5** (IC₅₀ = 32 and 38 μ g/mL) was increased in their peracetylated derivative **11** (IC₅₀ = 16 μ g/mL). Since a glycoside with the same aglycon as **4** and **5** but with L-galactose instead of D-arabinose⁴ was shown to be inactive against *P. falciparum* (unpublished results), we proceeded to the synthesis and evaluation of the antiplasmodial activity of the arabinopyranosides **12–18**. In this series, only the perbenzoylated compounds **12** and **13** were active against *P. falciparum* (Table 1), compound **13** (IC₅₀ = 10 μ g/mL) being more active than the natural arabinopyranosides **4** and **5**. We also tested the antiplasmodial activity of simple sugar derivatives with the same stereochemistry as that of D-arabinopyranose, such as the D-fucosides **19** and **20** and D-galactosides **21** and **22**. Curiously, compounds **19–21** displayed antiplasmodial activity, while the perbenzoylated methyl β -D-galactoside **22** was inactive (Table 1). The whole series of natural and synthetic compounds mentioned above were also tested for their activity against intracellular *T. cruzi* (Table 1).¹⁵ In this assay, glycosides **4** and **5** and the elemene **6** displayed biological activity (46, 48, and 45 μ g/mL, respectively). The antitrypanosomal activity of glycosides **4** and **5** was increased when these compounds were peracetylated (compound **11** showed an IC₅₀ = 15.9 μ g/mL).

Given the antiplasmodial activity displayed by natural tyramine derivatives (**1–3**), we proceeded to synthesize and evaluate the antiplasmodial activity of compounds **23–35** (Table 2). Of this series, the derivatives with a fatty acid moiety (**28–31**) showed antiplasmodial activity very similar to those of their natural analogues. Variations in the structure and activity of the natural and synthetic tyramides suggested that increasing the number of carbons of the fatty acid chain produces an increase in potency (**28–30**), while the presence of polar groups on the fatty acid chain decreases potency (**31**). On the other hand, the introduction of bromine atoms on the tyramine aromatic ring (**35**) produced an increase in the antiplasmodial activity. Finally, the activity displayed by compound **32** (IC₅₀ = 8 μ g/mL) indicated that the change in

Table 2. Activity of Compounds **23**–**35** against Chloroquine-Resistant *Plasmodium falciparum*^a

compound	<i>P. falciparum</i> , IC ₅₀ (μM)
23	160
24	>50
25	>50
26	64
27	>50
28	72
29	47
30	34
31	62
32	24
33	117
34	>50
35	17
chloroquine	0.07

^a ND = not determined.

the position of the amide bond (see **29** for comparison purposes) also produces an increase in antimalarial activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter using a 1 dm path length cell. CD spectra were obtained in MeCN in a JASCO J-720 spectropolarimeter. NMR spectra were recorded in *d*-chloroform at 250, 400, or 500 MHz using a Bruker DPX-250, Varian Inova-400, and Bruker AMX-500, respectively (*d*-chloroform as internal standard). HREIMS were measured on a Micromass Autospec mass spectrometer. CC was performed on Si gel 60, 70–230 mesh for VLC and 230–400 mesh for flash columns. Semipreparative HPLC purification was carried out on a Waters apparatus equipped with a refractive index detector and a spherisorb 10 μm Si semiprep column (10 × 250 mm).

Biological Material. Specimens of *M. austera* (voucher C5) were collected by scuba in Coiba National Park at 3–10 m depth in August 2002. The samples were frozen immediately after collection and stored at –80 °C until extraction. Voucher specimens are maintained for reference at the Smithsonian Tropical Research Institute. Specimen C5 was consistent with the original description for *M. austera* (Verrill, 1869):¹⁶ large reddish or yellowish brown colonies, up to 30 cm in height and thicker dichotomous branches 6–12 mm in diameter, with occasional branching anastomosis near the base, and rough surface. Calyces are prominently conical, having an acute lower lip, and made by coarse and large light orange spindle sclerites (1.25–1.70 mm in length) encompassing the polyp aperture. Sclerites are predominantly reddish, with some white spindles. Largest sclerites are blunt and stout (0.65–1.45 mm), mostly oblong or oval in shape covered by rough warts, and smaller sclerites are short, irregular and thorny spindles (0.15–0.30 mm).

Extraction and Isolation. The octocoral *M. austera* was cut into small pieces and extracted with MeOH and CH₂Cl₂. The extracts were pooled and concentrated under reduced pressure in a rotary evaporator to yield 21.0 g of crude extract, which was subjected to solvent partition, affording four fractions (*n*-hexane, CH₂Cl₂, *n*-butanol, and H₂O), which were submitted to biological assays. The antiplasmodial activity was located in the *n*-hexane and CH₂Cl₂ fractions. The active CH₂Cl₂ fraction (2.2 g) was subjected to activity-guided fractionation using VLC eluted with CH₂Cl₂–MeOH mixtures of increasing polarity (from 3% to 67% of MeOH), yielding five fractions. Fraction 2 (517 mg) was purified by two successive Si gel flash chromatographies (column A, CH₂Cl₂–MeOH, 2% to 9% MeOH, four fractions; column B, CH₂Cl₂–MeOH, 2% MeOH, seven fractions). The sixth fraction from column B (165 mg) was subjected to normal-phase HPLC purification (isooctane–EtOAc, 1:1, 1 mL/min, isocratic elution), yielding compounds **1** (12 mg), **2** (5 mg), and **3** (6 mg). The seventh fraction from column B was subjected to Si gel column chromatography (column C, CH₂Cl₂–acetone, 6% to 50% acetone, eight fractions) to yield compounds **4** (2 mg) and **5** (3 mg) in fractions 8 and 9, respectively. The *n*-hexane fraction (6.3 g), also active in the antiplasmodial assay, was subjected to VLC (3% to 50% of EtOAc in *n*-hexane), yielding eight fractions. Fraction 2 was subjected to normal-phase HPLC purification (2.5% EtOAc in isooctane, 1 mL/min, isocratic elution), yielding compounds **6** (10 mg), **7** (6 mg), and **8** (2 mg).

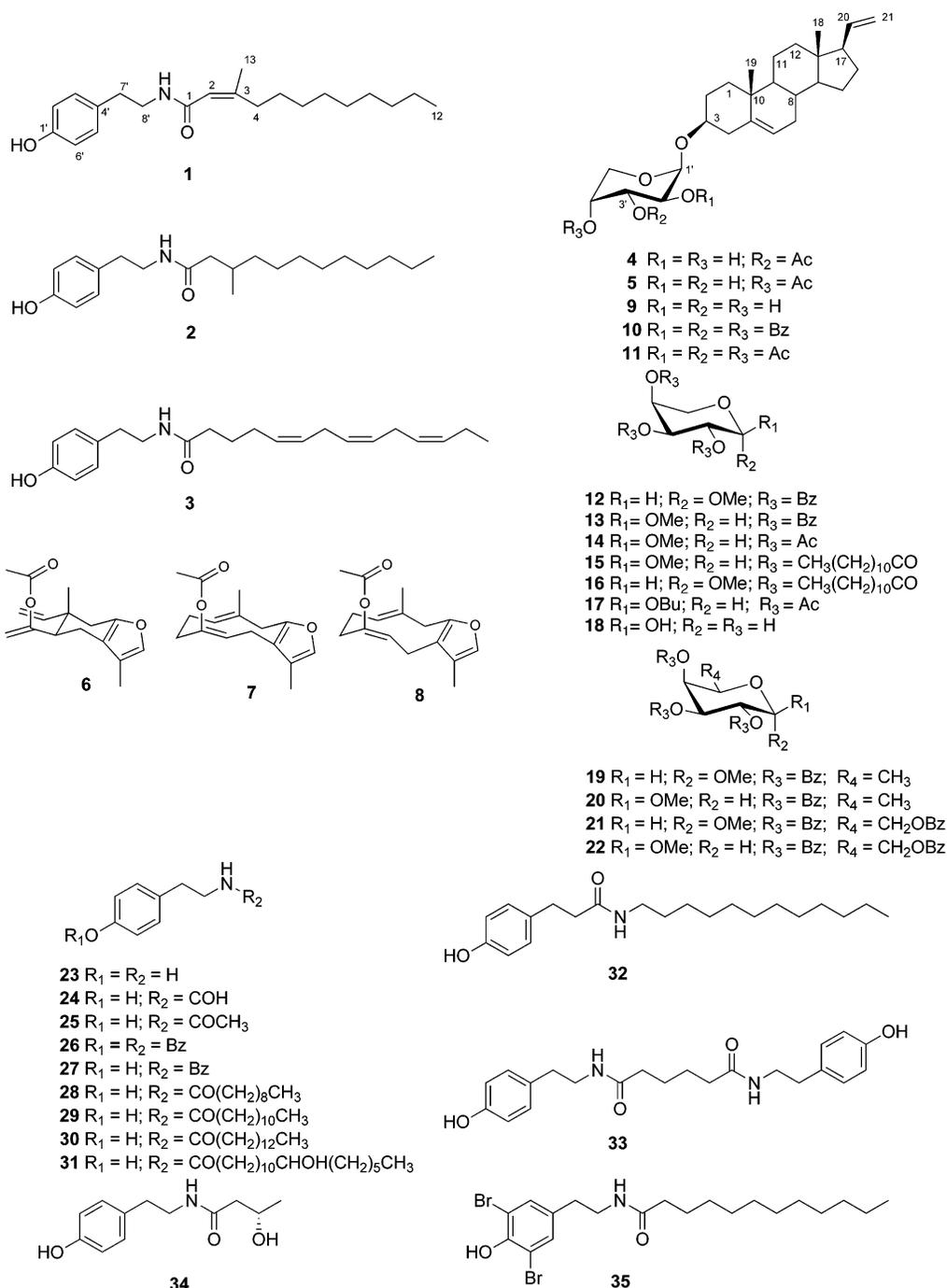
N-(4-Hydroxyphenethyl)-3-methyldodecanamide (2): oil; [α]_D²⁵ –26.8 (c 0.5, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 7.03 (2H, d, *J* = 8.4 Hz, H-3', H-5'), 6.78 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 5.44 (1H, bt, NH), 3.48 (2H, dd, *J* = 12.0, 6.8 Hz, H-8'), 2.73 (2H, t, *J* = 6.8 Hz, H-7'), 2.12 (1H, m, H-2a), 1.87 (1H, m, H-3), 1.86 (1H, m, H-2b), 1.26 (2H, m, H-4), 1.25 (14H, bs, H-5 to H-11), 0.87 (3H, m, H-13), 0.88 (3H, m, H-12); ¹³C NMR (CDCl₃, 100 MHz) δ 172.9 (C, C-1), 154.7 (C, C-1'), 130.4 (C, C-4'), 129.8 (2 CH, C-3', C-5'), 115.5 (2 CH, C-2', C-6'), 44.7 (CH₂, C-2), 40.7 (CH₂, C-8'), 36.8 (CH₂, C-4), 34.8 (CH₂, C-7'), 31.8 (CH₂, C-10), 30.7 (CH, C-3), 29.8* (CH₂, C-5), 29.7* (CH₂, C-6), 29.6* (CH₂, C-7), 29.3* (CH₂, C-8), 26.9* (CH₂, C-9), 22.7 (CH₂, C-11), 19.6 (CH₃, C-13), 14.1 (CH₃, C-12); EIMS *m/z* 330 (0.4), 282 (2), 214 (12), 157 (6), 129 (1), 120 (100), 106 (23), 91 (3), 77 (4), * these signals may be interchanged; HRESI-TOFMS *m/z* 356.2562 (calcd for C₂₁H₃₅NO₂Na, 356.2560).

(5Z,8Z,11Z)-N-(4-Hydroxyphenylethyl)tetradeca-5,8,11-trienamide (3): oil; ¹H NMR (CDCl₃, 400 MHz) δ 7.02 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 6.79 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 5.49 (1H, bt, NH), 5.35 (6H, m, H-5, H-6, H-8, H-9, H-11, H-12), 3.48 (2H, dd, *J* = 12.0, 7.0 Hz, H-8'), 2.78 (2H dd, *J* = 11.8, 5.8 Hz, H-7), 2.78 (4H dd, *J* = 11.8, 5.8 Hz, H-10), 2.73 (2H, t, *J* = 7.0 Hz, H-7'), 2.14 (2H, m, H-2), 2.08 (2H, m, H-4), 2.04 (2H, m, H-13), 1.68 (2H, m, H-3), 0.96 (3H, t, *J* = 7.6 Hz, H-14); ¹³C NMR (CDCl₃, 100 MHz) δ 173.2 (C, C-1), 154.8 (C, C-1'), 132.1 (CH, C-12), 130.2 (C, C-4'), 129.7 (2 CH, C-3', C-5'), 128.9 (CH, C-5), 128.8 (CH, C-6), 128.5 (CH, C-9), 127.9 (CH, C-8), 126.9 (CH, C-11), 115.5 (2 CH, C-2', C-6'), 40.8 (CH₂, C-8'), 36.1 (CH₂, C-2), 34.7 (CH₂, C-7'), 26.6 (CH₂, C-4), 25.59 (CH₂, C-10), 25.52 (CH₂, C-7), 25.49 (CH₂, C-3), 20.54 (CH₂, C-13), 14.3 (CH₃, C-14); EIMS *m/z* 341 [M]⁺ (0.6), 192 (1), 120 (100), 107 (38), 77 (10); HRESI-TOFMS *m/z* 364.2231 (calcd for C₂₂H₃₁NO₂Na, 364.2250).

3'-O-Acetyl-3β-pregna-5,20-dienyl-β-D-arabinopyranoside (4): white powder; [α]_D²⁵ –98.3 (c 0.515, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 5.76 (1H, ddd, *J* = 16.2, 10.9, 7.7, H-20), 5.36 (1H, d, *J* = 5.2 Hz, H-6), 5.09 (1H, dd, *J* = 3.2, 10.1 Hz, H-3'), 5.06 (1H, d, *J* = 3.8 Hz, H-1'), 4.99 (1H, d, *J* = 16.2 Hz, H-21a), 4.96 (1H, d, *J* = 10.9 Hz, H-21b), 4.03 (1H, bs, H-4'), 3.95 (1H, m, H-2'), 3.95 (1H, m, H-5'), 3.67 (1H, dd, *J* = 12.5, 2.1 Hz, H-5'b), 3.52 (1H, m, H-3), 2.36 (1H, ddd, *J* = 13.0, 4.9, 2.1 Hz, H-4a), 2.23 (1H, m, H-4b), 2.18 (3H, s, H-7'), 2.01 (1H, m, H-7a), 1.95 (1H, m, H-17), 1.81 (1H, m, H-16a), 1.89 (1H, m, H-1a), 1.88 (1H, m, H-2a), 1.71 (1H, m, H-8), 1.69 (2H, m, H-12), 1.67 (1H, m, H-15), 1.61 (1H, m, H-2b), 1.56 (1H, m, H-16b), 1.55 (1H, m, H-7b), 1.52 (2H, m, H-11a), 1.48 (1H, m, H-11b), 1.19 (1H, m, H-15b), 1.07 (1H, m, H-1b), 1.02 (1H, m, H-14), 1.02 (3H, s, H-19), 0.97 (1H, m, H-9), 0.61 (3H, s, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ 170.8 (C, C-6'), 140.16 (C, C-5), 139.8 (CH, C-20), 122.2 (CH, C-6), 114.5 (CH₂, C-21), 97.5 (CH, C-1'), 77.8 (CH, C-3), 73.0 (CH, C-3'), 68.3 (CH, C-4'), 67.2, (CH, C-2'), 62.4 (CH₂, C-5'), 55.9 (CH, C-14), 55.3 (CH, C-17), 50.4 (CH, C-9), 43.4 (C, C-13), 38.4 (CH₂, C-4), 37.4 (CH₂, C-12), 37.3 (CH₂, C-1), 36.8 (C, C-10), 31.9 (CH, C-8), 31.9 (CH₂, C-7), 29.7 (CH₂, C-2), 27.2 (CH₂, C-16), 24.8 (CH₂, C-15), 21.1 (CH₃, C-7'), 20.6 (CH₂, C-11), 19.4 (CH₃, C-19), 12.7 (CH₃, C-18); COSY (selected) H-3 (H-2a, H-2b, H-4a, H-4b), H-6 (H-7), H-20 (H-21a, H-21b, H-17), H-1 (H-2'), H-2' (H-1', H-3'), H-3' (H-2', H-4'), H-4' (H-3', H-5a', H-5b'), H-5a' (H-5b', H-4'), H-5b' (H-5a, H-4'); HMBC H-18 (C-12, C-13, C-14), H-19 (C-1, C-5, C-9, C-10), H-21 (C-17), H-1' (C-3, C-5', C-3'), H-2' (C-4'), H-3' (C-2', C-6'), H-5' (C-1), H-7' (C-6'); EIMS *m/z* 396 [M – HOAc – H₂O] (0.01), 342 (2), 282 (100), 267 (22), 213 (9), 175 (5), 115 (3), 91 (2); EIMS *m/z* 396 [M – HOAc – H₂O] (0.01), 342 (2), 282 (100), 267 (22), 213 (9), 175 (5), 115 (3), 91 (2); HRESI-TOFMS *m/z* 497.2846 (calcd for C₂₈H₄₂O₆Na, 497.2874).

4'-O-Acetyl-3-pregna-5,20-dienyl-β-D-arabinopyranoside (5): white powder; [α]_D²⁵ –205.0 (c 0.16, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 5.76 (1H, ddd, *J* = 16.5, 10.9, 7.8, H-20), 5.36 (1H, d, *J* = 5.0 Hz, H-6), 5.15 (1H, d, *J* = 3.4 Hz, H-4'), 5.05 (1H, d, *J* = 3.9 Hz, H-1'), 4.98 (1H, d, *J* = 16.5 Hz, H-21a), 4.95 (1H, d, *J* = 10.9 Hz, H-21b), 3.92 (1H, m, H-3'), 3.91 (1H, m, H-5'a), 3.78 (1H, dd, *J* = 9.6, 3.9 Hz, H-2'), 3.71 (1H, dd, *J* = 13.0, 2.1 Hz, H-5'b), 3.49 (1H, m, H-3), 2.36 (1H, ddd, *J* = 13.1, 4.7, 2.1 Hz, H-4a), 2.23 (1H, m, H-4b), 2.15 (3H, s, H-7'), 2.03 (1H, m, H-7a), 1.95 (1H, m, H-17), 1.87 (1H, m, H-1a), 1.88 (1H, m, H-2a), 1.81 (1H, m, H-16a), 1.71 (2H, m, H-12), 1.71 (1H, m, H-8), 1.69 (1H, m, H-15a), 1.59 (1H, m, H-11a), 1.58 (1H, m, H-2b), 1.57 (1H, m, H-7b), 1.56 (1H, m, H-16b), 1.49 (1H, m, H-11b), 1.19 (1H, m, H-15b), 1.08 (1H, dd, *J* = 12.1, 3.6 Hz, H-1b), 1.02 (3H, s, H-19), 1.01 (1H, m, H-14), 0.96 (1H, m, H-9), 0.61 (3H,

Chart 1



s, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ 170.8 (C, C-6'), 139.9 (C, C-5); 139.6 (CH, C-20), 122.1 (CH, C-6), 114.5 (CH₂, C-21), 97.0 (CH, C-1'), 77.9 (CH, C-3), 69.8 (CH, C-3'), 71.2 (CH, C-4'), 69.2, (CH, C-2'), 60.9 (CH₂, C-5'), 55.9 (CH, C-14), 55.3 (CH, C-17), 50.4 (CH, C-9), 43.4 (C, C-13), 38.7 (CH₂, C-4), 37.4 (CH₂, C-12), 37.3 (CH₂, C-1), 36.9 (C, C-10), 32.0 (CH, C-8), 32.0 (CH₂, C-7), 29.6 (CH₂, C-2), 27.3 (CH₂, C-16), 24.9 (CH₂, C-15), 21.2 (CH₃, C-7'), 20.7 (CH₂, C-11), 19.5 (CH₃, C-19), 12.8 (CH₃, C-18); COSY (selected) H-3 (H-2a, H-2b, H-4a, H-4b), H-6 (H-7), H-20 (H-21a, H-21b, H-17), H-1' (H-2'), H-2' (H-1', H-3'), H-3' (H-2', H-4'), H-4' (H-3', H-5a', H-5b'), H-5a (H-5b', H-4'), H-5b' (H-5a', H-4'); HMBC H-4 (C-2, C-3, C-5, C-6, C-10), H-6 (C-4, C-8, C-10), H-18 (C-12, C-13, C-14, C-17), H-19 (C-1, C-5, C-9, C-10), H-21 (C-17, C-20), H-1' (C-3, C-3'), H-3' (C-1', C-4'), H-5' (C-1', C-3', C-4'), H-7' (C-6'); EIMS *m/z* 396 [M - HOAc - H₂O] (0.01), 342 (9), 300 (4), 282 (100), 267 (41), 213 (24), 175 (40), 115 (56), 91 (36), 43 (64); HRESI-TOFMS *m/z* 497.2858 (calcd for C₂₈H₄₂O₆Na, 497.2874).

Antiplasmodial Assay. The antiplasmodial activity was determined in a chloroquine-resistant *P. falciparum* strain (W2) utilizing a novel microfluorimetric assay to measure the inhibition of the parasite growth based on the detection of the parasitic DNA by intercalation with PicoGreen.¹⁴ *P. falciparum* was cultured according to the methods described by Trager and Jensen.¹⁷ The parasites were maintained at 2% hemeatocrit in flat-bottom flasks (75 mL) with RPMI 1640 medium (GibcoBRL) supplemented with 10% human serum.

Intracellular *T. cruzi* Assay. The recombinant Tulahuén clone C4 of *Trypanosoma cruzi* that expresses β-galactosidase (β-Gal) as a reporter enzyme was used in the assay (Buckner et al., 1996).¹⁵ The method is based on the growth inhibition effect of test samples on trypomastigote, the intracellular form of the parasite, infecting Vero cells. The resulting color from the cleavage of chlorophenol red-β-D-galactoside (CPRG) by β-Gal expressed by the parasite was determined in a Benchmark plate reader (BIO-RAD) employing a 570 nm wavelength filter. Antitrypanosomal activity was expressed as IC₅₀,

which is a measure of the concentration of test substance that inhibits 50% parasite growth on duplicate samples, as compared with an untreated control. Assays were conducted at 37 °C under an atmosphere of 5% CO₂ and 95% air mixture.

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Supporting Information Available: General procedures for methanolysis and for the synthesis of sugar esters and tyramine derivatives; experimental details for compounds **10–17**, **19–22**, **24–35**; and CD spectra of compounds **10** and **12**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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