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Oxysterols from an octocoral of the genus *Gorgonia* from the eastern Pacific of Panama†

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Eighteen new oxysterols have been isolated from a previously undescribed octocoral collected from the eastern Pacific of Panama. Their structures were determined based on spectroscopic evidence. The absolute configuration was established by derivatization with (R)- and (S)-MPA. Antimicrobial and antileishmanial effects were evaluated.

Introduction

Sterols play an ecological role in marine organisms as substances in chemical defense against predators and competitor reef organisms. They also exhibit diverse biological activities, *e.g.*, cytotoxicity, carcinogenesis, atherogenicity, hypocholesterolemia, mutagenesis and liver X receptor agonist (LXR).¹ Certain oxysterols can regulate proteins involved in cholesterol efflux through activation of LXR, which is one means by which oxysterols affect cholesterol homeostasis.^{2,3} Steroids are a major group of secondary metabolites of soft corals and, among them, the subclass Octocorallia, particularly the sub-orders Holaxonia and Alcyoniina, produces the largest number of polyhydroxy sterols.^{4–6} Most corals subject to chemical studies have been collected in the Indo Pacific, Caribbean and Red Sea, and very few on the Pacific side of Mesoamerica.^{7–9} This paper presents eighteen new oxysterols from an octocoral of the genus *Gorgonia* collected from the eastern Pacific coast of Panama. These compounds bear the same cholestane nucleus but display different oxidation patterns on the ring system.

All eighteen oxysterols show oxidations at C-3, C-5, C-6 and C-22. Some of them exhibit additional oxidations on the steroidal nucleus and at C-25 on the side chain. Based on the

different positions of oxidation, this set of steroids can be divided into two groups as depicted in Fig. 1 (groups A and B). All steroids of group A have also been oxidized at C-19 and are distributed into two series: 3 α -oxysteroids (1–11), and 3 β -oxysteroids (12–14), three of them bear a C-19–C-6 oxygen bridge (9–11). The remaining 15–18 compose group B. Compound 15 contains an extra oxidation position at C-9. Compound 16 possesses four oxidized carbons in the steroidal nucleus (C-3, C-5, C-6 and C-9) and conserves the C-19 methyl group. Compound 17 does not have any extra oxidation positions at the steroidal nucleus. Finally, compound 18 is the sole oxysterol of the 3 α -series that conserves the C-19 methyl group and presents additional oxidations at C-9 and C-11.

Results and discussion

From the crude extract of *Gorgonia* sp. collected off Aleta Island (Panama), compounds 1–18 were obtained after reversed-phase flash chromatography followed by gel filtration and successive HPLC.

Compound 1 was isolated as an amorphous solid [α]_D²⁰ –49 (*c* 0.35, CH₂Cl₂). Its HREIMS showed a peak at 494.3622 which corresponds to the empirical formula C₂₉H₅₀O₆ [M]⁺, indicating five degrees of unsaturation. Absorptions for hydroxyl and carbonyl groups at 3420 and 1716 cm^{–1} were observed in the IR spectrum. The ¹³C NMR and DEPT spectra of 1 (Table 1) showed the presence of 29 carbon signals assigned to 5 × CH₃ (one from an acetyl group), 11 × CH₂ (including one attached to hydroxyl group), 9 × CH (three bearing oxygen) and 4 quaternary carbons (one carbonyl, and one sp³ attached to oxygen). The analysis of ¹H and ¹³C NMR data indicates that 1 must be a cholestane steroid with five oxidation sites. 2D NMR experiments confirmed its structure and allowed to place the three hydroxyl groups and the acetoxy group in the molecule. HMBC correlations of H₂-19 with C-1, C-5, C-9 and C-10 indicate that the characteristic Me-19 of the cholestane skeleton is oxidized to

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Fig. 1 New steroids isolated from *Gorgonia* sp.

a hydroxymethyl group. Also HMBC correlations of H-3 with C-5 established the presence of hydroxyl groups at C-3 and C-5 and the HMBC correlation of H-22 with C-21 and C-24 indicated that the third hydroxyl group is placed at C-22. Finally the HMBC correlation of H-6 with the carbonyl at δ_C 169.6 ppm established the presence of an acetoxy group at C-6. Relative and absolute configuration will be discussed in later.

From accurate mass measurement, compound 2 was found to possess a molecular formula of $C_{31}H_{52}O_7$ (m/z 536.3731 $[M]^+$) with six degrees of unsaturation. The spectroscopic data of 2 were very similar to those of 1, the most significant differences

being the presence of signals indicative of a second acetoxy group (δ_C 21.4 and δ_C 169.0) and the chemical shift of C-3 (δ_H 5.30, br s; δ_C 71.2). These differences can be explained by the presence of an acetoxy instead of the hydroxyl group at C-3 found in compound 1.

Compound 3 was found to have the same molecular formula as 2 ($C_{31}H_{52}O_7$ (m/z 536.3688 $[M]^+$)). The spectroscopic data of 3 were very similar to those of 1, the most significant differences being the presence of signals indicative of a second acetoxy group (δ_H 2.02, s; δ_C 21.2 and δ_C 170.9) and the chemical shift of C-22 (δ_H 4.90, dd, $J = 7.6, 7.6$ Hz; δ_C 76.5). These differences can

Table 1 ^{13}C NMR (125 MHz) data for compounds 1–18 in CDCl_3

Pos.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	21.8	22.3	21.8	21.8	21.7	26.3	21.8	22.2	18.7	18.7	18.7	25.4	25.5	25.5	25.8	26.9	32.0	27.3
2	29.5	26.1	29.5	29.5	29.2	25.9	25.9	26.1	28.8	28.8	28.7	31.1	27.2	31.2	30.9	30.3 ^a	30.7	31.9
3	68.4	71.2	68.4	68.4	68.2	71.5	71.4	71.1	67.4	67.4	67.4	67.2	70.6	67.2	66.9	67.4	67.3	65.9
4	36.1	34.8	36.2	36.1	36.0	35.1	34.9	34.7	37.3	37.3	37.3	40.9	37.3	41.1	40.7	40.7	40.6	31.9
5	73.6	73.0	73.6	73.6	73.7	74.4	73.9	73.0	75.9	75.9	75.9	74.6	74.1	74.6	75.8	77.6	75.3	81.8
6	76.2	75.8	76.1	76.0	76.0	74.6	74.4	75.7	80.8	80.8	80.8	76.1	76.4	76.1	76.4	76.0	76.1	212.4
7	30.3	30.4	30.3	30.3	30.2	33.6	34.3	30.4	30.6	30.6	30.6	30.9	30.9	31.0	29.5	29.1	31.4	36.6
8	31.2	31.2	31.2	31.2	31.1	31.3	30.6	31.1	33.4	33.4	33.4	31.2	31.3	31.2	33.7	32.4	30.8	40.1
9	45.5	45.3	45.4	45.5	45.4	45.6	45.2	45.3	44.7	44.7	44.7	45.5	45.3	45.5	78.5	78.8	45.4	78.3
10	44.8	44.6	44.8	44.8	44.7	43.2	43.0	44.5	44.9	44.8	44.8	43.6	43.7	43.6	46.7	41.2	38.5	49.0
11	22.5	22.5	22.5	22.3	22.4	21.1	22.1	22.4	22.1	22.1	22.1	22.6	22.7	22.8	20.3	29.7 ^a	21.2	69.7
12	40.5	40.5	40.5	40.4	40.4	40.5	40.4	40.5	40.1	40.2	40.2	40.5	40.5	40.4	34.7	34.8	40.0	46.3
13	42.8	42.8	42.8	43.1	42.7	43.0	42.7	42.6	43.2	43.1	43.2	42.8	42.8	42.8	42.6	42.6	42.7	42.4
14	56.0	56.1	56.1	55.5	56.0	56.8	56.1	56.1	54.8	54.8	54.8	56.1	56.2	56.1	48.6	48.7	55.8	47.7
15	24.0	24.0	24.0	24.3	24.0	24.0	24.1	24.0	23.5	23.5	23.5	24.0	24.0	24.0	24.0	24.0	24.1	23.6
16	27.7	27.7	28.0	27.7	28.0	28.1	28.1	28.0	27.9	28.2	28.2	27.7	27.7	28.1	27.9	28.0	27.7	27.8
17	52.6	52.6	52.6	52.1	52.5	52.6	52.6	52.6	52.7	52.6	52.6	52.5	52.5	52.6	52.5	52.6	52.7	52.2
18	12.4	12.4	12.2	12.7	12.2	12.3	12.0	12.2	12.4	12.2	12.2	12.4	12.4	12.3	11.2	11.0	12.1	12.0
19	62.7	62.5	62.7	62.7	62.5	65.6	64.0	62.4	69.2	69.2	69.2	62.5	62.6	62.6	62.7	20.0	16.5	22.4
20	40.2	40.2	38.9	49.6	39.0	39.2	39.2	39.1	40.2	38.8	39.1	40.2	40.2	38.9	38.9	38.9	40.3	38.8
21	11.5	11.5	12.7	16.5	12.7	12.7	12.7	12.7	11.5	12.7	12.7	11.4	11.4	12.7	12.7	12.7	11.5	12.7
22	73.9	73.9	76.5	214.8	76.6	76.6	76.6	76.5	73.9	76.6	76.6	73.9	73.9	76.5	76.4	76.4	73.9	76.1
23	33.2	33.3	29.9	39.5	26.9	27.1	27.1	27.0	33.2	29.9	27.0	33.2	33.2	30.0	30.0	29.9	33.3	29.9
24	35.7	35.7	34.9	32.5	39.7	39.8	39.8	39.7	35.7	34.9	39.8	35.7	35.7	34.9	35.0	35.0	35.7	34.9
25	28.2	28.2	28.1	27.5	70.7	70.7	70.7	70.7	28.2	28.1	70.7	28.2	28.2	28.0	28.0	28.0	28.2	28.0
26	22.7	22.7	22.7	22.4	29.3	29.4	29.5	29.4	22.7	22.7	29.4	22.5	22.6	22.7	22.6	22.6	22.7	22.6
27	22.5	22.5	22.4	22.4	29.1	29.2	29.2	29.2	22.6	22.4	29.2	22.5	22.5	22.4	22.4	22.4	22.6	22.6
3 CO	—	169.0	—	—	—	169.1	169.0	168.9	—	—	—	—	170.7	—	—	—	—	—
3 COCH ₃	—	21.4	—	—	—	21.5	21.5	21.4	—	—	—	—	21.3	—	—	—	—	—
6 CO	169.6	169.5	169.5	169.6	169.7	—	—	169.5	—	—	—	169.9	169.6	169.8	169.4	—	170.1	—
6 COCH ₃	21.4	21.4	21.4	21.4	21.4	—	—	21.3	—	—	—	21.4	21.4	21.2	21.4	—	21.4	—
19 CO	—	—	—	—	—	—	171.0	—	—	—	—	—	—	—	—	—	—	—
19 COCH ₃	—	—	—	—	—	—	21.2 ^a	—	—	—	—	—	—	—	—	—	—	—
22 CO	—	—	170.9	—	171.1	171.0	170.9	171.0	—	170.9	170.9	—	—	171.0	170.8	171.2	—	170.8
22 COCH ₃	—	—	21.2	—	21.2	21.2	21.1 ^a	21.1	—	21.2	21.1	—	—	21.2	21.2	21.2	—	21.2

^a Interchangeable.

be explained by the presence of an acetoxy instead of the hydroxyl group at C-22 found in compound 1.

The molecular formula of 4 was determined as $\text{C}_{29}\text{H}_{48}\text{O}_6$ (m/z 492.3434 $[\text{M}]^+$). The spectroscopic data of 4 indicate that it possesses the same steroidal nucleus as 1. Therefore differences must be on the side chain that must contain a ketone (δ_{C} 214.8). HMBC correlation between the carbonyl group and H-20, H₃-21 and H₂-23 confirmed the presence of a ketone at C-22 instead of the hydroxyl group of 1.

Compound 5 possesses a molecular formula of $\text{C}_{31}\text{H}_{52}\text{O}_8$ (m/z 552.3671 $[\text{M}]^+$) with six degrees of unsaturation. Comparison of NMR data of 3 and 5 suggests that both compounds bear the same substituents and the same configuration on the steroidal nucleus. Differences appear on the side chain: a singlet at δ_{H} 1.19 s (6H) instead of the doublets of 3 and a quaternary carbon at δ_{C} 70.7 (C-25) instead of the methine of 3. These data indicate that 5 contains a hydroxyl group at C-25.

Molecular formula of 6 is the same as that of 5 ($\text{C}_{31}\text{H}_{52}\text{O}_8$ m/z 552.3589 $[\text{M}]^+$). Comparison of their NMR data indicates that they must be positional isomers. HMBC correlation of the carbonyl at δ_{C} 169.1 with H-3 (δ_{H} 5.32) and ^1H and ^{13}C NMR

chemical shifts of C-6 (δ_{H} 3.60, dd, $J = 2.8, 2.8$ Hz; δ_{C} 74.6) indicate that 6 contains a hydroxyl at C-6 and an acetoxy group at C-3.

The presence of three acetoxy groups in 7 is deduced from its molecular formula, $\text{C}_{33}\text{H}_{54}\text{O}_9$ (m/z 594.3768 $[\text{M}]^+$) and its ^{13}C NMR data (Table 1). From the comparison of the NMR data of 7 and 6 it can be deduced that 7 is the C-19 acetoxy derivative of 6 since the most significant differences are found at C-19 (δ_{H} 4.65, d, $J = 12.9$ Hz, 4.27, d, $J = 12.9$ Hz; δ_{C} 64.0) and it was confirmed by the HMBC correlation of the carbonyl at δ_{C} 171.0 with H₂-19.

The molecular formula $\text{C}_{33}\text{H}_{54}\text{O}_9$ (m/z 594.3769 $[\text{M}]^+$) and NMR data of 8 indicates that 8 and 7 are positional isomers. Chemical shifts of H-3 (δ_{H} 5.28, br s) and H-22 (δ_{H} 4.93, dd, $J = 6.3, 6.4$) indicated that positions C-3 and C-22 are acetylated in 8 as it occurs in 7. Chemical shifts of H-6 (δ_{H} 4.68, br s) and H₂-19 (δ_{H} 4.18, d, $J = 12.4$ Hz, 3.75, d, $J = 12.4$ Hz) together with the HMBC correlation of the carbonyl at δ_{C} 169.5 with H-6 established that the third acetoxy group is located at C-6.

Compound 9 was isolated as an amorphous solid. Its HREIMS showed a peak at m/z 434.3389 which corresponds to the empirical formula $\text{C}_{27}\text{H}_{46}\text{O}_4$, indicating five degrees of

unsaturation. In the IR spectrum absorptions for hydroxyl at 3416 cm^{-1} were observed but no absorption for carbonyl groups. From the ^1H and ^{13}C NMR (Tables 1 and 3) data it can be deduced that **9** is a steroid with positions C-3, C-5, C-6, C-19 and C-22 oxidized but its molecular formula only shows four oxygen atoms. Comparison of its NMR data with those of **1**, shows that the side chain is the same for both compounds and differences are found at C-6 and C-19. In **9** chemical shifts of C-6 and C-19 move downfield, and H_2 -19 coupling constants change from 12.6 Hz to 8.5 Hz, which indicate that a cyclic ether must connect C-6 and C-19 thus satisfying the five degrees of unsaturation of the molecular formula.

From accurate mass measurement, compound **10** was found to have a molecular formula of $\text{C}_{29}\text{H}_{48}\text{O}_5$ (m/z 499.3386 $[\text{M} + \text{Na}]^+$) with six degrees of unsaturation. Absorptions for hydroxyl and carbonyl groups at 3416 and 1731 cm^{-1} were observed in the IR spectrum. The spectroscopic data of **10** were very similar to those of **9**, the most significant differences being the presence of signals indicative of an acetoxy group (δ_{C} 21.1 and δ_{C} 170.9) and the chemical shift of C-22 (δ_{H} 4.91, dd, $J = 6.6, 6.9$ Hz; δ_{C} 76.6). These differences can be explained by the presence of an acetoxy group at C-22 instead of the hydroxyl group found in compound **9**.

Compound **11** possesses a molecular formula of $\text{C}_{29}\text{H}_{48}\text{O}_6$ (m/z 492.3455 $[\text{M}]^+$) with six degrees of unsaturation. Comparison of NMR data of **10** and **11** suggests that both compounds possess the same substituents and the same configuration on the steroidal nucleus. Differences appear on the side chain. Me-26 and Me-27 show as a singlet (δ_{H} 1.20 s [6H]) instead of the doublets of **10** and C-25 appears as a quaternary carbon at δ_{C} 70.7 instead of the methine of **10**. These data indicate that **11** contains another hydroxyl group at C-25.

There is a precedent of an oxysterol containing a C-19–C-6 oxygen bridge that has been obtained as the result of the spontaneous cyclization of its C-6/C-19 di-hydroxyl derivative.¹⁰ However, when a sample of **6** was stirred in CDCl_3 at room temperature for 30 days no changes were observed in the starting material and no ether derivative was detected.

Compound **12** possesses the same molecular formula as **1** $\text{C}_{29}\text{H}_{50}\text{O}_6$ (m/z 494.3627 $[\text{M}]^+$) and their ^1H and ^{13}C NMR data are very similar. The most significant difference is the shape of H-3 (br s) in **1** whereas in **12** it appears as a well resolved doublet of doublets (dddd, $J = 4.6, 4.6, 10.4, 10.4$) which indicates that both compounds are epimers at C-3.

The same relationship is deduced for the epimers at C-3 **13** and **2** and for the couple **3** and **14**.

Compound **15** was isolated as an amorphous solid whose molecular formula was established as $\text{C}_{31}\text{H}_{52}\text{O}_8$ by HREIMS m/z 552.3663 $[\text{M}]^+$, IR absorptions at 3394, 1732 and 1718 cm^{-1} indicated the presence of hydroxyl and acetoxy groups. The ^{13}C NMR and DEPT spectra of **15** (Table 1) showed the presence of 31 carbon signals assigned to $6 \times \text{CH}_3$ (two from acetoxy groups), $11 \times \text{CH}_2$ (one bonded to hydroxyl group), $8 \times \text{CH}$ (three attached to oxygen) and 6 quaternary carbons (two from acetoxy groups, and two sp^3 bearing oxygen). 1D and 2D NMR indicate that acetoxy groups are located at C-6 and C-22 and positions C-3, C-5 and C-19 are linked to hydroxyl groups.

HMBC correlation of the additional oxidized carbon at δ_{C} 78.5 ppm with H_2 -19 indicates that another tertiary hydroxyl group must be at C-9. This is the first example of a steroid with this oxidation pattern on the steroidal nucleus.

HRESIMS $[\text{M}]^+$ m/z 494.3580 established the molecular formula $\text{C}_{29}\text{H}_{50}\text{O}_6$ for **16**. IR absorptions at 3375, and 1731 cm^{-1} indicated the presence of hydroxyl and acetoxy groups. The ^{13}C NMR and DEPT spectra of **16** (Table 1) showed the presence of 29 carbon signals assigned to $6 \times \text{CH}_3$ (one from an acetoxy group), $10 \times \text{CH}_2$, $8 \times \text{CH}$ (three attached to oxygen) and 5 quaternary carbons (one from acetoxy group, and two sp^3 attached to oxygen). NMR data indicated the presence of the two methyl groups of the steroidal nucleus, H_3 -18 and H_3 -19. HMBC correlation of the carbonyl at δ_{C} 171.2 with H-22 (δ_{H} 4.91, dd, $J = 6.3, 7.2$ Hz) placed the acetoxy group at C-22. Therefore positions C-3, C-5, C-6 and C-9 are attached to hydroxyl groups. Thus the structure of **16** was established as represented in Fig. 1.

Molecular formula of **17** was established as $\text{C}_{29}\text{H}_{50}\text{O}_5$ (m/z 478.3641 $[\text{M}]^+$). The ^{13}C NMR and DEPT spectra of **17** (Table 1) showed the presence of 29 carbon signals assigned to $6 \times \text{CH}_3$ (one from an acetoxy group), $10 \times \text{CH}_2$, $9 \times \text{CH}$ (three bonded to oxygen) and 4 quaternary carbons (one from acetoxy group, and one sp^3 attached to oxygen). The spectroscopic data of **17** were very similar to those of **12**, indicating that they possess the same side chain and substituents of the steroidal nucleus at C-3, C-5 and C-6 remain the same. The most significant difference being the presence of a methyl group at δ_{C} 16.5; δ_{H} 1.14 (s) instead of the hydroxymethyl group found in **12** (δ_{C} 62.5; δ_{H} 4.20, d, $J = 12.3$ Hz, 3.89, d, $J = 12.3$ Hz). The presence of the methyl group at C-10 was confirmed by the HMBC correlations of H_3 -19 with C-1, C-5 and C-9. Therefore the structure of **17** was established as represented on Fig. 1.

Compound **18** was isolated as an amorphous solid. Its HREIMS showed a peak at m/z 508.3397 $[\text{M}]^+$ which corresponds to the empirical formula $\text{C}_{29}\text{H}_{48}\text{O}_7$, indicating six degrees of unsaturation. Absorptions for hydroxyl and carbonyl groups at 3438 and 1732 cm^{-1} were observed in the IR spectrum. The ^{13}C NMR and DEPT spectra of **18** (Table 1) showed the presence of 29 carbon signals assigned to $6 \times \text{CH}_3$ (one from an acetoxy group), $9 \times \text{CH}_2$, $8 \times \text{CH}$ (three bonded to oxygen) and 6 quaternary carbons (one ketone, one from an acetoxy group, and two sp^3 bearing oxygen). The comparison of its NMR data with those of the previously described steroids indicates that its side chain contains an acetoxy group at C-22. HMBC correlations of H_3 -18 with C-12, C-13, C-14 and C-17 together with the COSY correlation H-11/ H_2 -12 placed a hydroxyl group at C-11. HMBC correlations of H_3 -19 with C-1, C-5, C-9 and C-10 placed the tertiary hydroxyl groups at C-5 and C-9. Chemical shift of C-7 (δ_{C} 36.6) and H_2 -7 (δ_{H} 2.81, dd, $J = 13.5, 13.5$; 2.19, d, $J = 13.2$) together with the HMBC correlations of the ketone (δ_{C} 212.4) with H_2 -7 indicated that the ketone must be at C-6.

Relative configuration of the steroidal nucleus

The relative configurations of the 18 new steroids were established based on 2D NOESY experiments, molecular mechanics calculations and coupling constants. The fusion of the rings of

Table 2 ¹H NMR (500 MHz) data for compounds 1–6 in CDCl₃

	1	2	3	4	5	6
Pos.	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)
1	1.92 (m), 1.66 (m)	1.98 (m), 1.51 (m)	1.93 (m), 1.66 (m)	1.92 (m), 1.66 (m)	1.91 (m), 1.53 (m)	1.66 (m), 1.58 (m)
2	β : 1.81 (m), α : 1.75 (m)	β : 1.82 (m), α : 1.50 (m)	β : 1.84 (m), α : 1.76 (m)	β : 1.84 (m), α : 1.79 (m)	1.73 (m), 1.57 (m)	β : 1.84 (m), α : 1.76 (m)
3	4.29 (br s)	5.30 (br s)	4.29 (br s)	4.30 (br s)	4.26 (br s)	5.32 (br s)
4	β : 2.02 (dd, 3.5, 15.1), α : 1.57 (m)	β : 2.15 (dd, 3.4, 15.5), α : 1.55 (m)	β : 2.02 (m), α : 1.58 (m)	β : 2.03 (m), α : 1.59 (m)	β : 2.00 (m), α : 1.50 (m)	β : 2.60 (dd, 4.4, 15.8), α : 1.59 (m)
5	—	—	—	—	—	—
6	4.71 (br s)	4.70 (br s)	4.71 (br s)	4.71 (br s)	4.70 (br s)	3.60 (dd, 2.8, 2.8)
7	1.67 (m), 1.50 (m)	1.68 (m), 1.56 (m)	1.65 (m), 1.50 (m)	1.57 (m), 1.67 (m)	1.64 (m), 1.47 (m)	1.76 (m), 1.50 (m)
8	1.67 (m)	1.68 (m)	1.66 (m)	1.65 (m)	1.48 (m)	2.09 (m)
9	1.59 (m)	1.61 (m)	1.60 (m)	1.63 (m)	1.56 (m)	1.52 (m)
10	—	—	—	—	—	—
11	1.57 (m), 1.37 (m)	1.55 (m), 1.55 (m)	1.59 (m), 1.51 (m)	1.56 (m), 1.56 (m)	1.54 (m), 1.46 (m)	1.48 (m), 1.48 (m)
12	β : 1.98 (m), α : 1.17 (m)	β : 1.98 (m), α : 1.18 (m)	β : 1.99 (m), α : 1.13 (m)	β : 1.95 (m), α : 1.25 (m)	β : 1.97 (m), α : 1.13 (m)	β : 1.99 (m), α : 1.15 (m)
13	—	—	—	—	—	—
14	1.16 (m)	1.15 (m)	1.13 (m)	1.14 (m)	1.10 (m)	1.05 (m)
15	1.57 (m), 1.03 (dd, 6.9, 11.7)	1.54 (m), 1.04 (m)	1.53 (m), 1.00 (m)	1.50 (m), 1.10 (m)	1.55 (m), 1.01 (m)	1.55 (m), 1.05 (m)
16	1.88 (m), 1.26 (m)	1.89 (m), 1.23 (m)	1.91 (m), 1.23 (m)	1.75 (m), 1.25 (m)	1.86 (m), 1.48 (m)	1.89 (m), 1.22 (m)
17	1.42 (m)	1.42 (m)	1.18 (m)	1.60 (m)	1.15 (m)	1.16 (m)
18	0.72 (s)	0.73 (s)	0.71 (s)	0.74 (s)	0.71 (s)	0.71 (s)
19	4.18 (d, 12.6), 3.74 (d, 12.6)	4.19 (d, 12.3), 3.76 (d, 12.3)	4.17 (d, 12.6), 3.75 (d, 12.6)	4.17 (d, 12.6), 3.75 (d, 12.6)	4.16 (d, 12.6), 3.73 (d, 12.6)	4.01 (d, 12.3), 3.80 (d, 12.3)
20	1.39 (m)	1.39 (m)	1.52 (m)	2.49 (m)	1.52 (m)	1.52 (m)
21	0.86 (d, 6.6)	0.88 (d, 6.3)	0.94 (d, 6.6)	1.07 (d, 6.9)	0.95 (d, 6.9)	0.96 (d, 6.6)
22	3.60 (dd, 6.6, 6.6)	3.61 (dd, 6.0, 6.0)	4.90 (dd, 7.6, 7.6)	—	4.92 (dd, 7.3, 6.6)	4.95 (dd, 7.6, 7.6)
23	1.47 (m), 1.32 (m)	1.46 (m), 1.33 (m)	1.55 (m), 1.42 (m)	2.41 (dd, 6.3, 8.3), 2.35 (dd, 6.3, 9.1)	1.66 (m), 1.47 (m)	1.68 (m), 1.50 (m)
24	1.26 (m), 1.11 (m)	1.28 (m), 1.10 (m)	1.10 (m), 1.10 (m)	1.41 (m), 1.67 (m)	1.39 (m), 1.39 (m)	1.39 (m), 1.39 (m)
25	1.50 (m)	1.52 (m)	1.50 (m)	1.50 (m)	—	—
26	0.86 (d, 6.6)	0.86 (d, 6.3)	0.86 (d, 6.6)	0.87 (d, 6.6)	1.19 (s)	1.20 (s)
27	0.86 (d, 6.6)	0.88 (d, 6.3)	0.86 (d, 6.6)	0.87 (d, 6.6)	1.19 (s)	1.20 (s)
3 COCH ₃	—	2.07 (s) ^a	—	—	—	2.06 (s)
6 COCH ₃	2.05 (s)	2.06 (s) ^a	2.05 (s)	2.05 (s)	2.04 (s)	—
22 COCH ₃	—	—	2.02 (s)	—	2.02 (s)	2.03 (s)
OH	—	—	—	—	—	3.34 (br s)

^a Interchangeable.

Table 3 ¹H NMR (500 MHz) data for compounds 7–12 in CDCl₃

Pos.	7	8	9	10	11	12
	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)
1	2.05 (m), 1.57 (m)	1.97 (m), 1.50 (m)	1.56 (m), 1.39 (m)	1.54 (m), 1.40 (m)	1.55 (m), 1.39 (m)	2.05 (m), 1.44 (m)
2	β : 1.71 (m), α : 1.68 (m)	1.81 (m), 1.48 (m)	β : 1.72 (m), α : 1.55 (m)	β : 1.71 (m), α : 1.20 (m)	β : 1.72 (m), α : 1.23 (m)	1.88 (m), 1.44 (m)
3	5.31 (br s)	5.28 (br s)	4.29 (dd, 2.5, 2.5)	4.29 (br s)	4.29 (br s)	4.12 (dddd, 4.6, 4.6, 10.4, 10.4)
4	β : 2.48 (dd, 4.1, 15.7), α : 1.56 (m)	β : 2.15 (dd, 4.1, 15.7), α : 1.56 (m)	β : 1.89 (m), α : 1.26 (m)	β : 1.90 (m), α : 1.54 (m)	β : 1.90 (m), α : 1.23 (m)	1.84 (m), 1.62 (m)
5	—	—	—	—	—	—
6	3.55 (br s)	4.68 (br s)	3.67 (d, 4.4)	3.66 (d, 3.1)	3.66 (d, 3.8)	4.69 (br s)
7	1.71 (m), 1.46 (m)	1.66 (m), 1.52 (m)	1.54 (m), 1.51 (m)	1.56 (m), 1.51 (m)	1.54 (m), 1.51 (m)	1.66 (m), 1.56 (m)
8	1.71 (m)	1.63 (m)	1.58 (m)	1.56 (m)	1.54 (m)	1.90 (m)
9	1.62 (m)	1.60 (m)	1.72 (m)	1.71 (m)	1.70 (m)	1.38 (m)
10	—	—	—	—	—	—
11	1.54 (m), 1.50 (m)	1.53 (m), 1.48 (m)	1.39 (m), 1.13 (m)	1.37 (m), 1.13 (m)	1.38 (m), 1.13 (m)	1.55 (m), 1.54 (m)
12	β : 1.96 (m), α : 1.40 (m)	β : 1.98 (m), α : 1.13 (m)	β : 1.98 (ddd, 3.5, 3.5, 12.9), α : 1.26 (m)	β : 1.98 (m), α : 1.20 (m)	β : 1.98 (m), α : 1.15 (m)	β : 1.98 (m), α : 1.15 (m)
13	—	—	—	—	—	—
14	1.11 (m)	1.11 (m)	1.27 (m)	1.24 (m)	1.24 (m)	1.13 (m)
15	1.55 (m), 1.01 (m)	1.55 (m), 1.01 (m)	1.54 (m), 1.10 (m)	1.52 (m), 1.08 (m)	1.52 (m), 1.08 (m)	1.54 (m), 1.04 (m)
16	1.91 (m), 1.23 (m)	1.89 (m), 1.23 (m)	1.88 (m), 1.27 (m)	1.90 (m), 1.49 (m)	1.90 (m), 1.22 (m)	1.89 (m), 1.26 (m)
17	1.20 (m)	1.16 (m)	1.45 m	1.20 (m)	1.19 (m)	1.43 (m)
18	0.66 (s)	0.71 (s)	0.71 (s)	0.70 (s)	0.70 (s)	0.73 (s)
19	4.65 (d, 12.9), 4.27 (d, 12.9)	4.18 (d, 12.4), 3.75 (d, 12.4)	3.82 (d, 8.5), 3.72 (d, 8.5)	3.81 (d, 8.8), 3.72 (d, 8.8)	3.81 (d, 8.5), 3.72 (d, 8.5)	4.20 (d, 12.3), 3.89 (d, 12.3)
20	1.52 (m)	1.52 (m)	1.41 (m)	1.52 (m)	1.54 (m)	1.39 (m)
21	0.96 (d, 6.6)	0.95 (d, 6.7)	0.88 (d, 6.6)	0.94 (d, 6.9)	0.96 (d, 6.9)	0.88 (d, 6.6)
22	4.95 (dd, 7.2, 7.6)	4.93 dd (6.3, 6.4)	3.61 ddd (1.3, 5.4, 7.8)	4.91 dd (6.6, 6.9)	4.94 dd (7.2, 7.6)	3.60 dd (6.6, 5.7)
23	1.67 (m), 1.48 (m)	1.68 (m), 1.50 (m)	1.48 (m), 1.35 (m)	1.40 (m), 1.55 (m)	1.68 (m), 1.51 (m)	1.47 (m), 1.33 (m)
24	1.40 (m), 1.40 (m)	1.67 (m), 1.39 (m)	1.26 (m), 1.12 (m)	1.09 (m), 1.09 (m)	1.40 (m), 1.22 (m)	1.26 (m), 1.12 (m)
25	—	—	1.52 (m)	1.50 (m)	—	1.52 (m)
26	1.20 (s)	1.19 (s)	0.88 (d, 6.6)	0.86 (d, 6.6)	1.20 (s)	0.88 (d, 6.6)
27	1.20 (s)	1.19 (s)	0.88 (d, 6.6)	0.86 (d, 6.6)	1.20 (s)	0.88 (d, 6.6)
3 COCH ₃	2.07 (s)	2.06 (s)	—	—	—	—
6 COCH ₃	—	2.05 (s)	—	—	—	2.07 (s)
19 COCH ₃	2.03 (s) ^a	—	—	—	—	—
22 COCH ₃	2.04 (s) ^a	—	—	—	—	—
OH	3.40 (br s)	2.02 (s)	—	2.02 (s)	2.03 (s)	—

^a Interchangeable.

Table 4 ¹H NMR (500 MHz) data for compounds 13–18 in CDCl₃

Pos.	13	14	15	16	17	18
	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)
1	2.05 (m), 1.55 (m)	2.03 (m), 1.42 (m)	2.09 (m), 1.52 (m)	2.09 (m), 1.28 (m)	1.56 (m), 1.43 (m)	2.35 (d, 14.8), 1.80 (m)
2	β : 1.88 (m), α : 1.54 (m)	1.92 (m), 1.23 (m)	1.96 (m), 1.40 (m)	1.90 (m), 1.57 (m)	1.83 (m), 1.50 (m)	2.03 (m), 1.80 (m)
3	5.13 (dddd, 5.4, 5.4, 11.0, 11.0)	4.09 (dddd, 5.0, 5.0, 10.3, 10.3)	4.24 (dddd, 5.4, 5.4, 10.7, 10.7)	4.20 (dddd, 5.4, 5.4, 10.7, 10.7)	4.07 (dddd, 6.0, 6.0, 11.4, 11.4)	4.09 (br s)
4	β : 1.84 (m), α : 1.69 (m)	β : 1.84 (m), α : 1.61 (m)	1.70 (m), 1.66 (m)	1.98 (m), 1.58 (m)	1.83 (m), 1.56 (m)	1.97 (m), 1.24 (m)
5	—	—	—	—	—	—
6	4.65 (br s)	4.68 (br s)	4.74 (br s)	3.60 (br s)	4.69 (br s)	—
7	1.70 (m), 1.54 (m)	1.64 (m), 1.54 (m)	1.90 (m), 1.43 (m)	1.90 (m), 1.38 (m)	1.60 (m), 1.54 (m)	2.81 (dd, 13.5, 13.5)
8	1.70 (m)	1.71 (m)	2.11 (m)	2.15 (m)	1.60 (m)	2.19 (d, 13.2)
9	1.46 (m)	1.37 (m)	—	—	1.30 (m)	1.93 (m)
10	—	—	—	—	—	—
11	1.55 (m), 1.47 (m)	1.60 (m), 1.54 (m)	n.d.	n.d.	1.33 (m), 1.40 (m)	4.27 (dd, 5.3, 10.0)
12	β : 1.98 (m), α : 1.18 (m)	β : 1.98 (m), α : 1.12 (m)	1.80 (m), 1.40 (m)	β : 1.79 (m), α : 1.38 (m)	β : 1.98 (m), α : 1.20 (m)	2.03 (m), 1.44 (m)
13	—	—	—	—	—	—
14	1.14 (m)	1.08 (m)	1.58 (m)	1.54 (m)	1.11 (m)	1.80 (m)
15	1.55 (m), 1.01 (m)	1.54 (m), 1.01 (dd, 4.0, 6.9)	1.50 (m), 1.04 (m)	1.53 (m), 1.53 (m)	1.54 (m), 1.03 (dd, 6.3, 13.8)	1.53 (m), 1.03 (m)
16	1.53 (m), 1.26 (m)	1.91 (m), 1.22 (m)	1.96 (m), 1.24 (m)	n.d.	1.88 (m), 1.28 (m)	1.80 (m), 1.25 (m)
17	1.47 (m)	1.16 (m)	1.25 (m)	1.25 (m)	1.43 (m)	1.28 (m)
18	0.71 (s)	0.71 (s)	0.74 (s)	0.70 (s)	0.68 (s)	0.66 (s)
19	4.21 (d, 12.6), 3.89 (d, 12.6)	4.18 (d, 12.5), 3.87 (d, 12.5)	4.06 (d, 13.2), 3.97 (d, 13.2)	1.25 (s)	1.14 (s)	0.93 (s)
20	1.37 (m)	1.50 (m)	1.51 (m)	1.51 (m)	1.40 (m)	1.50 (m)
21	0.88 (d, 6.6)	0.93 (d, 6.6)	0.94 (d, 6.8)	0.94 (d, 6.6)	0.88 (d, 6.6)	0.96 (d, 6.9)
22	3.60 (dd, 7.0, 7.2)	4.91 (dd, 6.6, 7.1)	4.91 (dd, 7.1, 7.2)	4.91 (dd, 6.3, 7.2)	3.60 (dd, 6.6, 5.3)	4.89 (dd, 6.6, 7.1)
23	1.47 (m), 1.34 (m)	1.56 (m), 1.38 (m)	1.55 (m), 1.39 (m)	1.54 (m), 1.27 (m)	1.48 (m), 1.34 (m)	1.55 (m), 1.39 (m)
24	1.26 (m), 1.11 (m)	1.09 (m), 1.09 (m)	1.10 (m), 1.10 (m)	1.24 (m), 1.10 (m)	1.26 (m), 1.11 (m)	1.20 (m), 1.14 (m)
25	1.50 (m)	1.50 (m)	1.50 (m)	1.50 (m)	1.50 (m)	1.50 (m)
26	0.88 (d, 6.6)	0.85 (d, 6.6)	0.86 (d, 6.6)	0.86 (d, 6.6)	0.88 (d, 6.6)	0.86 (d, 6.6)
27	0.88 (d, 6.6)	0.85 (d, 6.6)	0.86 (d, 6.6)	0.86 (d, 6.6)	0.88 (d, 6.6)	0.86 (d, 6.6)
3 COCH ₃	2.00 (s)	—	—	—	—	—
6 COCH ₃	2.07 (s)	2.06 (s)	2.06 (s)	—	—	—
22 COCH ₃	—	2.01 (s)	2.02 (s)	2.03 (s)	—	2.03 (s)

the steroidal nucleus was established as all *trans* due to the observed NOEs between H₃-18 and H₃-19 (or H₂-19) with H-8 for all the 18 compounds. Therefore the six member rings must adopt a chair conformation. Except for **18**, in all compounds, the H-6 signal appears either as a br s or with small coupling constants (≈ 2.8 –4.4 Hz), which indicates that H-6 must be on equatorial disposition and, therefore the substituent at C-6 must be β .

On the other hand, attending to the H-3 coupling constants, we can differentiate compounds in two series: β and α . The α series composes **1**–**11** and **18**, with the substituent (hydroxyl or acetoxy group) at C-3 in α disposition, featuring H-3 as a br s or as a dd ($J = 2.5, 2.5$ Hz). Compounds **12** and **15**–**17** belong to the β series, with the substituent at C-3 in β disposition, as corroborated the well differentiated coupling constants of H-3 (≈ 5 and 10 Hz).

Also, in **18** the NOEs observed between H-11 and H₃-18 and H₃-19 indicate that the hydroxyl group at C-11 is located equatorial, on the opposite side of the molecule of Me-18 and Me-19.

Absolute configuration of the steroidal nucleus

Derivatization of **14** with (*R*)- and (*S*)- α -methoxy- α -phenylacetic acids (MPA) produced the diesters **14a** and **14b**. NMR analysis of the $\Delta\delta$ values of the protons influenced by the MPA group at C-3 of **14a** and **14b** gave clear evidence to assign the absolute configuration at C-3 as *S*, Table 5.

Absolute configuration of the side chain

The absolute configuration of C-22 of compound **13** was established by derivatization with (*R*)- and (*S*)-MPA which produced diesters **13a** and **13b**. NMR analysis of the $\Delta\delta$ values of the protons influenced by the MPA group at C-22 of **13a** and **13b** gave clear evidence to assign the absolute configuration at C-22 as *S*, Table 6. The observed NOEs between H₃-18 and H-20 indicate a β disposition for the side chain. Also, NOEs observed between H-22 and H₂-16 and between H₃-21 and H-12 β indicated C-21 must be in α disposition, therefore, considering that the absolute configuration at C-22 has been established as *S*, the absolute configuration of C-20 must be *S*. Based on these results, and considering that the 18 new steroids were isolated from the same organism, we can deduce the absolute configuration of the 18 steroids as shown on Fig. 2.

Studies on the biosynthesis origin of marine sterols are scarce,¹¹ and they are complicated by the fact that several sources contribute, for example, sterol absorption produced by

Table 6 ¹H NMR $\Delta\delta(\delta_R - \delta_S)$ values (CDCl₃, ppm, recorded at 500 MHz) of the MPA esters **13a** and **13b**

Pos.	δ_R	δ_S	$\Delta\delta$
H-17	0.98	0.94	+0.04
H ₃ -18	0.53	0.18	+0.35
H-20	1.45	1.28	+0.17
H ₃ -21	0.92	0.72	+0.20
H-23a	1.36	1.58	−0.22
H-23b	1.24	1.40	−0.16
H-25	1.32	1.42	−0.10
H ₃ -26	0.68	0.86	−0.18
H ₃ -27	0.71	0.86	−0.15

symbiotic organisms, assimilation or modification of sterols obtained from nutrition and biosynthesis *de novo*.¹²

In this study, the octocoral yielded compounds **1**–**18**, and they are a good example of how nature generates structural complexity and biological activity, by diversifying oxidation processes from a precursor of low oxidation state. It appears that these compounds are linked together by a network of enzymatic oxygenation processes around the active sites of normal nuclei 3 β -hydroxy- Δ^5 (ring A/B system) and side chains of eight carbon atoms, to give 3 β ,5 α ,6 β -; 3 β ,5 α ,6 β -19-oxy- and also isomerization to 3 α ,5 α ,6 β -19-oxy-sterols derivatives. Δ^5 activation of the allylic position is an important way of providing polar sterols, and it has been argued that its biosynthesis in octocorals follows a different path to the sponges.⁶ While in sponges the presence of unsaturation to spread oxidation in cascade toward the other end (rings C/D system) is necessary, their biogenesis in soft corals seems to run independently of the unsaturation activation pathway. Indeed, even when the Δ^5 is saturated or oxidized to epoxide or diols, oxidation of the rest of the carbons in the nucleus can proceed further.

Compound **17** features a previously known cholestane nuclear oxidation pattern with several examples of compounds found in corals among other organisms.^{13,14} Oxisterols **1**–**8** and **12**–**14** feature a nuclear oxidation pattern only found in five compounds previously isolated from corals,^{10,15–21} and the same occurs with compounds **16** and **18**, whose nuclear oxidation models have been described once for each case and both isolated from octocorals.^{22–24} **15** is the first example of a steroid with this oxidation pattern on the steroidal nucleus suggesting that these specific compounds are only produced by certain species, which may represent a chemical signature to these.

Antimicrobial activity

The antimicrobial activities of the steroids **1**, **3**, **4**, **6**, **8**–**10** and **13**–**15** were tested *in vitro* by the broth macrodilution method. Unfortunately, almost all tested compounds were found to be practically inactive against all bacterial strains under study with the exception of compounds **14** and **15** which showed weak activity. Compound **14** displayed weak antibacterial activity against Gram + *Bacillus cereus* and *Staphylococcus aureus* and Gram – *Klebsiella pneumoniae* with MIC values ranging from 25 to 50 $\mu\text{g mL}^{-1}$ on the three strains. Compound **15** showed activity against *K. pneumoniae* with a MIC $\geq 50 \mu\text{g mL}^{-1}$.

Table 5 ¹H NMR $\Delta\delta(\delta_R - \delta_S)$ values (CDCl₃, ppm, recorded at 500 MHz) of the MPA esters **14a** and **14b**

Pos.	δ_R	δ_S	$\Delta\delta$
H-2 β	0.78	1.36	−0.58
H-2 α	1.32	1.87	−0.55
H-4 β	1.85	1.77	+0.08
H-4 α	1.58	1.48	+0.1
H-6	4.58	4.53	+0.05

Fig. 2 Selected NOEs and coupling constants for compounds 1–18.

Antileishmanial effect

Antileishmanial effect of steroids 1–5, 7–11 and 14–18 was determined against *Leishmania infantum* promastigotes. Initial screening yielded highly variable results. 4, 5, 7–9, 11, 16–18 were the least active compounds ($\leq 55\%$ inhibition) whereas 14 reduced the multiplication of promastigotes by more than 95%, at 100 μM and compounds 1 and 2 reduced the multiplication of promastigotes by more than 70% at 100 μM . Compounds 3, 10 and 15 reduced the multiplication of promastigotes by 63, 56 and 60%, respectively at 100 μM (Table 7). Dose-response curves of the most active compounds showed IC_{50} values of 59.5 and 50.7 μM for 1 and 14, respectively.

Toxicity for mammalian cells (primary mouse peritoneal macrophages) (M ϕ) was explored for 1, 2, 3, 10, 14 and 15 using MTT method. No clear dose-response curve was obtained with the concentrations used and wide variations were found. Thus 50 μM of 2 reduced M ϕ viability by ca. 50% whereas the same concentration of 10 induced a mere 12% reduction in M ϕ

viability. Interestingly one of the compounds with the highest antileishmanial activity 14 did not provoke a 50% reduction with 400 μM . The high IC_{50} (>50 μM) found for compound 14 against *Leishmania* promastigotes compared to that of the positive control, amphotericin B ($\text{IC}_{50} = 0.1$ μM), probably excludes this steroid for antileishmanial monotherapy. However, the apparent reduced toxicity for mammalian cells suggests the interest of evaluating the antileishmanial efficacy of 14 against intracellular amastigotes and its potential synergistic effect with other well established antileishmanial drugs.

The relative activity of 1 and 3 compared with that of 14 indicates that compounds belonging to the β series may present better antileishmanial effect (Fig. 3). Compounds 1, 3 and 4, belong to α series and they possess the same functional groups and configurations at C-3, C-5, C-6 and C-19 but differ at C-22. From the analysis of their chemical structures it can be deduced that the presence of a hydroxyl group at C-22 increases the antileishmanial effect in this type of compounds, for

Table 7 Antileishmanial effect of compounds **1–5**, **7–11** and **14–18** in *Leishmania infantum* promastigotes

Compd	% inhibition		
	Concentration (μM)		
	10 μM	50 μM	100 μM
1	10.7	27.2	72.6
2	25.8	48.5	72.6
3	0.0	25.4	63.6
4	0.0	0.0	39.1
5	3.3	3.1	53.9
7	0.0	22.6	32.3
8	1.5	9.4	42.1
9	16.6	4.3	7.8
10	5.2	47.1	56.4
11	0.0	23.7	40.0
14	9.6	70.9	96.3
15	0.0	34.7	62.2
16	0.0	0.0	32.5
17	0.0	0.0	43.4
18	0.0	55.4	31.1

instance **1**. Moreover, it seems that the formation of the cyclic ether between C-6 and C-19 reduces the activity (tandem **1/9**). From comparison of **14** and **15** it can be deduced that hydroxylation of C-9 reduces the antileishmanial activity.

Experimental

General experimental procedures

Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 20 °C. IR spectra were recorded on a Perkin-Elmer 1650/FTIR spectrometer. ^1H NMR

and ^{13}C NMR, HSQC, HMBC and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^1H NMR and at 125 MHz for ^{13}C NMR. All ^{13}C and ^1H NMR spectra were internally referenced to the residual solvent signal (CDCl_3 ; δ_{C} 77.0 ppm, δ_{H} 7.25 ppm). Two-dimensional NMR spectra were obtained using the standard Bruker software. EIMS and HRMS data were taken on a Micromass Autospec spectrometer. ESIMS and HRESI MS (positive-ion mode) data were taken on a Micromass LCT Premier XE. The gel filtration column (Sephadex LH-20) used hexane/ CH_2Cl_2 /MeOH (3 : 1 : 1) as eluent. HPLC separations were performed on an Agilent 1200 Series Quaternary LC system using a Jaigel-Sil-043-10 semi-preparative column (10 μm , 20 $\times$ 250 mm) eluted with hexane/EtOAc mixtures. The spray reagent for TLC was $\text{H}_2\text{SO}_4/\text{H}_2\text{O}/\text{AcOH}$ (1 : 4 : 20).

Biological material

Gorgonia sp. was collected by SCUBA diving off Aleta Island (Panama) at -15 m.

Extraction

Air-dried samples (499 g) were extracted with acetone at room temperature, and were concentrated to give a dark residue (8.35 g).

Purification

Crude extract was fractionated by reversed-phase C-18 silica flash chromatography using step-gradient elution (100% H_2O to 100% MeOH). Fraction eluted with MeOH (100%) was subjected to a gel filtration chromatography (Sephadex LH-20) followed by HPLC to afford **1** (7.0 mg), **2** (3.2 mg), **3** (9.9 mg), **4** (1.8 mg), **5** (20.6 mg), **6** (3.8 mg), **7** (2.4 mg), **8** (59.6 mg), **9** (0.8 mg), **10** (3.0

Fig. 3 Structure and antileishmanial effect of some of the isolated compounds.

mg), **11** (0.8 mg), **12** (7.0 mg), **13** (7.3 mg), **14** (34.7 mg), **15** (4.8 mg), **16** (0.4 mg), **17** (3.0 mg) and **18** (2.0 mg).

Characterisation of compounds

Compound 1. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -49 (c 0.35, CH_2Cl_2); IR (film) ν_{max} 3420, 2944, 1716, 1258, 1031 cm^{-1} ; EIMS m/z 494 $[\text{M}]^+$ (55), 476 $[\text{M} - \text{H}_2\text{O}]^+$ (75), 458 $[\text{M} - 2\text{H}_2\text{O}]^+$ (25), 440 $[\text{M} - 3\text{H}_2\text{O}]^+$ (13), 426 $[\text{M} - \text{CH}_3\text{OH} - 2\text{H}_2\text{O}]^+$ (22); HREIMS $[\text{M}]^+$ m/z 494.3622 (calcd for $\text{C}_{29}\text{H}_{50}\text{O}_6$, 494.3607), 476.3499 (calcd for $\text{C}_{29}\text{H}_{48}\text{O}_5$, 476.3502), 458.3409 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_4$, 458.3396), 440.3307 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_3$, 440.3290), 426.3134 (calcd for $\text{C}_{28}\text{H}_{42}\text{O}_3$, 426.3134); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 2. Isolated as an amorphous solid; $[\alpha]_D^{20}$ $+58$ (c 0.12, CH_2Cl_2); IR (film) ν_{max} 3486, 2940, 1740, 1731, 1241, 1031 cm^{-1} ; EIMS m/z 536 $[\text{M}]^+$ (14), 458 $[\text{M} - \text{AcOH} - \text{H}_2\text{O}]^+$ (58), 367 $[\text{M} - 2\text{AcOH} - \text{CH}_2\text{OH} - \text{H}_2\text{O}]^+$ (14); HREIMS $[\text{M}]^+$ m/z 536.3731 (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_7$, 536.3713), 458.3418 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_4$, 458.3396), 367.3002 (calcd for $\text{C}_{26}\text{H}_{39}\text{O}$, 367.3001); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 3. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -27 (c 0.46, CH_2Cl_2); IR (film) ν_{max} 3420, 2947, 1732, 1716, 1245, 1031 cm^{-1} ; EIMS m/z 536 $[\text{M}]^+$ (10), 504 $[\text{M} - \text{CH}_3\text{OH}]^+$ (20), 458 $[\text{M} - \text{AcOH} - \text{H}_2\text{O}]^+$ (39), 440 $[\text{M} - \text{AcOH} - 2\text{H}_2\text{O}]^+$ (27), 409 $[\text{M} - \text{AcOH} - \text{CH}_2\text{OH} - 2\text{H}_2\text{O}]^+$ (30), 398 $[\text{M} - 2\text{AcOH} - \text{H}_2\text{O}]^+$ (25), 380 $[\text{M} - 2\text{AcOH} - 2\text{H}_2\text{O}]^+$ (23), 367 $[\text{M} - 2\text{AcOH} - \text{CH}_2\text{OH} - \text{H}_2\text{O}]^+$ (15); HREIMS $[\text{M}]^+$ m/z 536.3688 (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_7$, 536.3713), 504.3470 (calcd for $\text{C}_{30}\text{H}_{48}\text{O}_6$, 504.3451), 458.3386 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_4$, 458.3396), 440.3300 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_3$, 440.3290), 409.3123 (calcd for $\text{C}_{28}\text{H}_{41}\text{O}_2$, 409.3107), 398.3173 (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_2$, 398.3185), 380.3064 (calcd for $\text{C}_{27}\text{H}_{40}\text{O}$, 380.3079), 367.3015 (calcd for $\text{C}_{26}\text{H}_{39}\text{O}$, 367.3001); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 4. Isolated as an amorphous solid; $[\alpha]_D^{20}$ $+59$ (c 0.18, CH_2Cl_2); IR (film) ν_{max} 3420, 2940, 1732, 1716, 1241 cm^{-1} ; EIMS m/z 492 $[\text{M}]^+$ (59), 460 $[\text{M} - \text{CH}_3\text{OH}]^+$ (17); HREIMS $[\text{M}]^+$ m/z 492.3434 (calcd for $\text{C}_{29}\text{H}_{48}\text{O}_6$, 492.3451), 460.3188 (calcd for $\text{C}_{28}\text{H}_{44}\text{O}_5$, 460.3189); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 5. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -34 (c 0.68, CH_2Cl_2); IR (film) ν_{max} 3412, 2944, 1731, 1714, 1246 cm^{-1} ; EIMS m/z 552 $[\text{M}]^+$ (4), 521 $[\text{M} - \text{CH}_2\text{OH}]^+$ (9), 517 $[\text{M} - \text{H}_2\text{O} - \text{OH}]^+$ (8), 492 $[\text{M} - \text{C}_3\text{H}_8\text{O}]^+$ (54), 485 $[\text{M} - \text{CH}_2\text{OH} - 2\text{H}_2\text{O}]^+$ (10); HREIMS $[\text{M}]^+$ m/z 552.3671 (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_8$, 552.3662), 521.3486 (calcd for $\text{C}_{30}\text{H}_{49}\text{O}_7$, 521.3478), 517.3537 (calcd for $\text{C}_{31}\text{H}_{49}\text{O}_6$, 517.3529), 492.3091 (calcd for $\text{C}_{28}\text{H}_{44}\text{O}_7$, 492.3087), 485.3281 (calcd for $\text{C}_{30}\text{H}_{45}\text{O}_5$, 485.3267); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 6. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -24 (c 0.28, CH_2Cl_2); IR (film) ν_{max} 3412, 2936, 1732, 1716, 1258 cm^{-1} ; EIMS m/z 552 $[\text{M}]^+$ (9), 534 $[\text{M} - \text{H}_2\text{O}]^+$ (11), 516 $[\text{M} - 2\text{H}_2\text{O}]^+$ (8), 502 $[\text{M} - \text{CH}_3\text{OH} - \text{H}_2\text{O}]^+$ (10), 456 $[\text{M} - \text{AcOH} - 2\text{H}_2\text{O}]^+$ (80), 424 $[\text{M} - \text{AcOH} - \text{CH}_3\text{OH} - 2\text{H}_2\text{O}]^+$ (15), 414 $[\text{M} - 2\text{AcOH} - \text{H}_2\text{O}]^+$ (22); HREIMS $[\text{M}]^+$ m/z 552.3589 (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_8$, 552.3662), 534.3571 (calcd for $\text{C}_{31}\text{H}_{50}\text{O}_7$, 534.3557), 516.3438 (calcd for $\text{C}_{31}\text{H}_{48}\text{O}_6$, 516.3451), 502.3311 (calcd for

$\text{C}_{30}\text{H}_{46}\text{O}_6$, 502.3294), 456.3224 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_4$, 456.3240), 424.2980 (calcd for $\text{C}_{28}\text{H}_{40}\text{O}_3$, 424.2977), 414.3132 (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_3$, 414.3134); ^{13}C and ^1H NMR see Tables 1 and 2.

Compound 7. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -173 (c 0.05, CH_2Cl_2); IR (film) ν_{max} 3442, 2940, 1729, 1714, 1376, 1246 cm^{-1} ; EIMS m/z 594 $[\text{M}]^+$ (97), 456 $[\text{M} - 2\text{AcOH} - \text{H}_2\text{O}]^+$ (57), 346 $[\text{M} - \text{C}_3\text{H}_6\text{O}_2 - 2\text{AcOH} - 3\text{H}_2\text{O}]^+$ (10); HREIMS $[\text{M}]^+$ m/z 594.3768 (calcd for $\text{C}_{33}\text{H}_{54}\text{O}_9$, 594.3768), 456.3232 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_4$, 456.3240), 346.2674 (calcd for $\text{C}_{26}\text{H}_{34}$, 346.2661); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 8. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -58 (c 1.16, CH_2Cl_2); IR (film) ν_{max} 3442, 2951, 1732, 1716, 1373, 1246 cm^{-1} ; EIMS m/z 594 $[\text{M}]^+$ (20), 456 $[\text{M} - 2\text{AcOH} - \text{H}_2\text{O}]^+$ (20), 425 $[\text{M} - 2\text{AcOH} - \text{CH}_2\text{OH} - \text{H}_2\text{O}]^+$ (73); HREIMS $[\text{M}]^+$ m/z 594.3769 (calcd for $\text{C}_{33}\text{H}_{54}\text{O}_9$, 594.3768), 456.3235 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_4$, 456.3240), 425.3036 (calcd for $\text{C}_{28}\text{H}_{41}\text{O}_3$, 425.3056); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 9. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -107 (c 0.18, CH_2Cl_2); IR (film) ν_{max} 3416, 2933, 1245, 1081 cm^{-1} ; EIMS m/z 434 $[\text{M}]^+$ (7), 416 $[\text{M} - \text{H}_2\text{O}]^+$ (52), 398 $[\text{M} - 2\text{H}_2\text{O}]^+$ (79), 384 $[\text{M} - \text{CH}_3\text{OH} - \text{H}_2\text{O}]^+$ (2), 380 $[\text{M} - 3\text{H}_2\text{O}]^+$ (9), 366 $[\text{M} - \text{CH}_3\text{OH} - 2\text{H}_2\text{O}]^+$ (9), 348 $[\text{M} - \text{CH}_3\text{OH} - 3\text{H}_2\text{O}]^+$ (4); HREIMS $[\text{M}]^+$ m/z 434.3389 (calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4$, 434.3396), 416.3291 (calcd for $\text{C}_{27}\text{H}_{44}\text{O}_3$, 416.3290), 398.3165 (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_2$, 398.3185), 384.3047 (calcd for $\text{C}_{26}\text{H}_{40}\text{O}_2$, 384.3028), 380.3097 (calcd for $\text{C}_{27}\text{H}_{40}\text{O}$, 380.3079), 366.2921 (calcd for $\text{C}_{26}\text{H}_{38}\text{O}$, 366.2923), 348.2805 (calcd for $\text{C}_{26}\text{H}_{36}$, 348.2817); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 10. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -50 (c 0.14, CH_2Cl_2); IR (film) ν_{max} 3416, 2933, 1731, 1246, 1022 cm^{-1} ; EIMS m/z 416 $[\text{M} - \text{AcOH}]^+$ (68), 349 $[\text{M} - \text{AcOH} - \text{CH}_2\text{OH} - 2\text{H}_2\text{O}]^+$ (20), 304 $[\text{M} - \text{C}_{10}\text{H}_{20}\text{O}_2]^+$ (93); HRESIMS $[\text{M} + \text{Na}]^+$ m/z 499.3386 (calcd for $\text{C}_{29}\text{H}_{48}\text{O}_5\text{Na}$, 499.3399); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 11. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -300 (c 0.08, CH_2Cl_2); IR (film) ν_{max} 3408, 2933, 1731, 1248 cm^{-1} ; EIMS m/z 492 $[\text{M}]^+$ (15), 474 $[\text{M} - \text{H}_2\text{O}]^+$ (7); HREIMS $[\text{M}]^+$ m/z 492.3455 (calcd for $\text{C}_{29}\text{H}_{48}\text{O}_6$, 492.3451), 447.3354 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_5$, 474.3345); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 12. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -73 (c 0.35, CH_2Cl_2); IR (film) ν_{max} 3408, 2947, 1721, 1261, 1031 cm^{-1} ; EIMS m/z 494 $[\text{M}]^+$ (91), 476 $[\text{M} - \text{H}_2\text{O}]^+$ (41), 458 $[\text{M} - 2\text{H}_2\text{O}]^+$ (56), 440 $[\text{M} - 3\text{H}_2\text{O}]^+$ (85), 385 $[\text{M} - \text{AcOH} - \text{CH}_2\text{OH} - \text{H}_2\text{O}]^+$ (7), 366 $[\text{M} - \text{AcOH} - \text{CH}_3\text{OH} - 2\text{H}_2\text{O}]^+$ (6); HREIMS $[\text{M}]^+$ m/z 494.3627 (calcd for $\text{C}_{29}\text{H}_{50}\text{O}_6$, 494.3607), 476.3487 (calcd for $\text{C}_{29}\text{H}_{48}\text{O}_5$, 476.3502), 458.3406 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_4$, 458.3396), 440.3275 (calcd for $\text{C}_{29}\text{H}_{44}\text{O}_3$, 440.3290), 385.3110 (calcd for $\text{C}_{26}\text{H}_{41}\text{O}_2$, 385.3107), 366.2918 (calcd for $\text{C}_{26}\text{H}_{38}\text{O}$, 366.2923); ^{13}C and ^1H NMR see Tables 1 and 3.

Compound 13. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -50 (c 0.34, CH_2Cl_2); IR (film) ν_{max} 3442, 2947, 1721, 1259, 1031 cm^{-1} ; EIMS m/z 536 $[\text{M}]^+$ (4), 458 $[\text{M} - \text{AcOH} - \text{H}_2\text{O}]^+$ (8), 398 $[\text{M} - 2\text{AcOH} - \text{H}_2\text{O}]^+$ (2), 367 $[\text{M} - 2\text{AcOH} - \text{CH}_2\text{OH} - \text{H}_2\text{O}]^+$ (23); HREIMS $[\text{M}]^+$ m/z 536.3699 (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_7$, 536.3713), 458.3406 (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_4$, 458.3396), 398.3183 (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_2$, 398.3185), 367.2985 (calcd for $\text{C}_{26}\text{H}_{39}\text{O}$, 367.3001); ^{13}C and ^1H NMR see Tables 1 and 4.

Compound 14. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -54 (c 0.63, CH₂Cl₂); IR (film) $\nu_{\max}$ 3412, 2955, 1732, 1716, 1373, 1245, 1033 cm⁻¹; EIMS m/z 536 [M]⁺ (2), 500 [M - 2H₂O]⁺ (6), 458 [M - AcOH - H₂O]⁺ (29); HRESMS [M]⁺ m/z 536.3734 (calcd for C₃₁H₅₂O₇, 536.3713), 500.3505 (calcd for C₃₁H₄₈O₅, 500.3502), 458.3403 (calcd for C₂₉H₄₆O₄, 458.3396); ¹³C and ¹H NMR see Tables 1 and 4.

Compound 15. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -91 (c 0.48, CH₂Cl₂); IR (film) $\nu_{\max}$ 3394, 1732, 1718, 1245, 1035 cm⁻¹; EIMS m/z 552 [M]⁺ (24), 534 [M - H₂O]⁺ (17), 516 [M - 2H₂O]⁺ (26), 502 [M - CH₃OH - H₂O]⁺ (30); HREIMS [M]⁺ m/z 552.3663 (calcd for C₃₁H₅₂O₈, 552.3662), 534.3582 (calcd for C₃₁H₅₀O₇, 534.3557), 516.3427 (calcd for C₃₁H₄₈O₆, 516.3451), 502.3291 (calcd for C₃₀H₄₆O₆, 502.3294); ¹³C and ¹H NMR see Tables 1 and 4.

Compound 16. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -240 (c 0.04, CH₂Cl₂); IR (film) $\nu_{\max}$ 3375, 1731, 1714, 1243, 1057 cm⁻¹; EIMS m/z 494 [M]⁺ (18) 477 [M - OH]⁺; HRESIMS [M]⁺ m/z 494.3580 (calcd for C₂₉H₅₀O₆, 494.3607), 477.3597 (calcd for C₂₉H₄₉O₅, 477.3580); ¹³C and ¹H NMR see Tables 1 and 4.

Compound 17. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -263 (c 0.08, CH₂Cl₂); IR (film) $\nu_{\max}$ 3531, 1716, 1243, 1072 cm⁻¹; EIMS m/z 478 [M]⁺ (15), 400 [M - AcOH - H₂O]⁺ (31); HREIMS [M]⁺ m/z 478.3641 (calcd for C₂₉H₅₀O₅, 478.3658), 400.3342 (calcd for C₂₇H₄₄O₂, 400.3341); ¹³C and ¹H NMR see Tables 1 and 4.

Compound 18. Isolated as an amorphous solid; $[\alpha]_D^{20}$ -209 (c 0.14, CH₂Cl₂); IR (film) $\nu_{\max}$ 3438, 2955, 1732, 1246 cm⁻¹; EIMS m/z 508 [M]⁺ (18), 490 [M - H₂O]⁺ (8); HREIMS [M]⁺ m/z 508.3397 (calcd for C₂₉H₄₈O₇, 508.3400), 490.3300 (calcd for C₂₉H₄₆O₆, 490.3294); ¹³C and ¹H NMR see Tables 1 and 4.

(R)- and (S)-MPA ester derivatives 14a and 14b

A solution of compound **14** (4.1 mg, 7.6×10^{-3} mmol) in 0.5 mL of CH₂Cl₂ was treated with *N,N'*-dicyclohexylcarbodiimide (13.5 mg, 6.5×10^{-2} mmol), 4-dimethylaminopyridine (6.7 mg, 5.5×10^{-2} mmol) and (*R*)- α -methoxy- α -phenylacetic acid (12.7 mg, 7.6×10^{-2} mmol) and stirred at room temperature for 7 h. After filtration, the reaction mixture was purified by silica gel chromatography (hexane-EtOAc (1 : 1)) to give the (*R*)-MPA ester derivative **14a** (6.0 mg, 7.1×10^{-3} mmol, 93.4% yield). The same experimental procedure was followed to obtain the (*S*)-MPA ester derivative **14b** (6.7 mg, 8.0×10^{-3} mmol, 81.3% yield).

(R)- and (S)-MPA ester derivatives 13a and 13b

A solution of compound **13** (2.2 mg, 4.1×10^{-3} mmol) in 0.5 mL of CH₂Cl₂ was treated with *N,N'*-dicyclohexylcarbodiimide (7.3 mg, 3.5×10^{-2} mmol), 4-dimethylaminopyridine (3.6 mg, 2.9×10^{-2} mmol) and (*R*)- α -methoxy- α -phenylacetic acid (6.8 mg, 4.1×10^{-2} mmol) and stirred at room temperature for 7 h. After filtration, the reaction mixture was purified by silica gel chromatography (hexane-EtOAc (1 : 1)) to give the (*R*)-MPA ester derivative **13a** (1.9 mg, 2.2×10^{-3} mmol, 53.7% yield). The same experimental procedure was followed to obtain the (*S*)-MPA ester derivative **13b** (2.1 mg, 2.5×10^{-3} mmol, 61.6% yield).

Antimicrobial activity

Antimicrobial susceptibility test was performed by the broth macrodilution method (within the range 10–100 $\mu\text{g mL}^{-1}$) against the following strains obtained from the Spanish Collection of Type Cultures (CECT; Faculty of Biological Sciences, University of Valencia, Spain) and American Type Culture Collection (ATCC, USA): *Staphylococcus aureus* (ATCC 6538), *Salmonella* sp. (CECT 456), *Klebsiella pneumoniae* (ATCC 23357), *Escherichia coli* (ATCC 9637), *Bacillus cereus* (ATCC 21772), *Proteus mirabilis* (CECT 170), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCCMYA-2876) as described elsewhere.⁷ In the case of the yeast *C. albicans* the tryptic soy medium was replaced by the non-filament-inducing medium; YPD [2% (w/v) Bacto peptone, 1% (w/v) yeast extract and 2% (w/v) glucose]. The selected strains were chosen for key characteristics, in terms of ecology, physiology, metabolism and for ease of screening. Minimum inhibitory concentrations (MIC), was defined as the lowest substance concentration that completely inhibits microbial growth. MIC was determined from two independent experiments performed in triplicate for each concentration. All compounds were previously dissolved in DMSO.

Antileishmanial effect

Antileishmanial efficacy was determined using an isolate from *Leishmania infantum* (MCAN/ES/2001/UCM9) originally obtained from a naturally infected dog. Promastigotes were routinely cultured in RPMI 1640 medium (Lonza) with 10% heat-inactivated fetal calf serum, 100 U mL⁻¹ penicillin + 100 $\mu\text{g mL}^{-1}$ streptomycin + 1% L-glutamine and buffered with 20 mM HEPES at 26 °C.²⁵ In the preliminary screening promastigotes (1.25×10^6 promastigotes per mL) were exposed to 10 μM , 50 μM and 100 μM of each steroid. To determine the approximate IC₅₀ selected molecules were tested at 10 μM , 20 μM , 40 μM , 60 μM and 80 μM . Assays were performed in 96 well microtiter plates (Costar, Corning) at least in triplicate. Both positive (untreated promastigotes) and negative (promastigotes treated with 0.2 μM amphotericin B) were included. Plates were incubated at 26 °C under CO₂/air (5%/95%) atmosphere. *Leishmania* proliferation was determined by Alamar Blue method and the intensity of fluorescence (λ 550 excitation, λ 590 emission) measured. IC₅₀ was calculated by least squares fit. Macrophage (M ϕ) viability was determined using MTT method. Briefly, peritoneal M ϕ were obtained from BAL/c mice and cultured (3.75×10^6 cells per mL) (RPMI medium) in 96 well flat-bottomed plates (Costar, Corning) in a final volume of 200 μL for 24 h under CO₂/air atmosphere. Cells were exposed to 12.5 μM , 25 μM , 50 μM , 100 μM , 200 μM and 400 μM from each molecule for 24 h. Cellular viability was estimated with MTT²⁶ and absorbance read at 570 nm. Experiments were done in triplicate.

Conclusions

During the course of our chemical investigation we identified eighteen new oxysterols. These compounds bear the same

cholestane nucleus but display a different oxidation pattern on the rings system. Among them, compound **15** is the first example of a steroid with this oxidation pattern on the steroidal nucleus.

The antileishmanial effect of the steroids tested was moderate against promastigotes. Toxicity for mammalian cells suggests the potential interest of the least toxic and most active molecule (**14**) in combination with other antileishmanial drugs.

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