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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 evidences. 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 of chemical defense against predators and competitors 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–B). All steroids of group A have also been oxidized at C-19 and are distributed into two series: 3α–oxysteroids (**1**–**11**), and 3β–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 3α–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 $[\alpha]^{20}_{D}$ –49 (*c* 0.35, CH_2Cl_2). Its HREIMS showed a peak at 494.3622 which corresponds to the empirical formula $C_{29}H_{50}O_6$ $[M]^+$, indicating five degrees of unsaturation. Absorptions for hydroxyl and carbonyl groups at 3420 and 1716 cm^{-1} were observed in the IR spectrum. The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed the presence of 29 carbon signals assigned to 5×CH_3 (one from an acetyl group), 11×CH_2 (including one attached to hydroxyl group), 9×CH (three bearing oxygen) and 4 quaternary carbons (one carbonyl, and one sp^3 attached to oxygen). The analysis of ^1H and ^{13}C 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 H_2-19 with C-1, C-5, C-9 and C-10 indicate that the characteristic Me-19 of the cholestane skeleton is oxidized to a hydroxymethyl group. Also HMBC correlations of H-3 with C-5 established the presence of

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Electronic Supplementary Information (ESI) available: ^1H and ^{13}C NMR spectra of compounds 1-18. See DOI: 10.1039/x0xx00000x

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

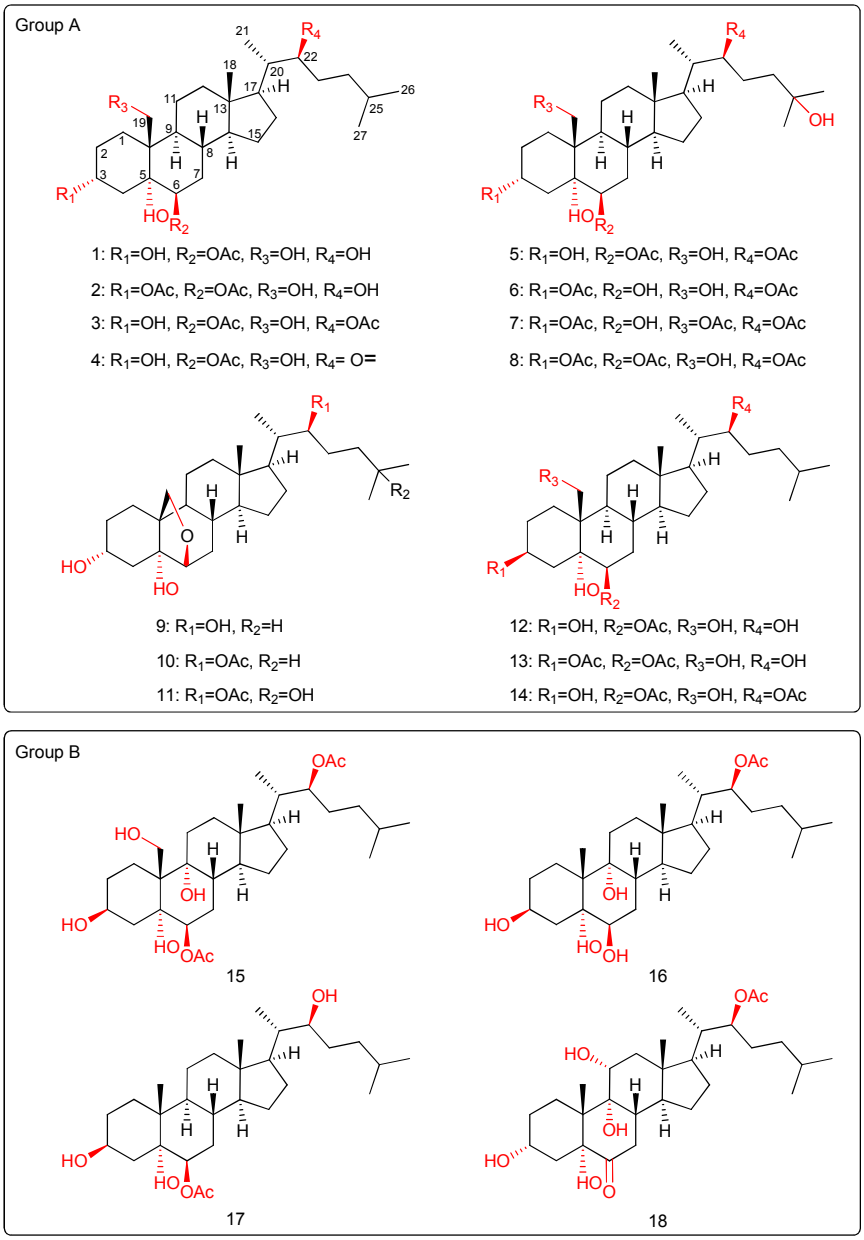

Fig. 1 New steroids isolated from *Gorgonia* sp.

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The molecular formula of **4** was determined as $C_{29}H_{48}O_6$ (*m/z* 492.3434 $[M]^+$). 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, H_3-21 and H_2-23 confirmed the presence of a ketone at C-22 instead of the hydroxyl group of **1**.

Compound **5** possesses a molecular formula of $C_{31}H_{52}O_8$ (*m/z* 552.3671 $[M]^+$) 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.

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*	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*	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 CO*CH$_3$*	---	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 CO*CH$_3$*	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 CO*CH$_3$*	---	---	---	---	---	---	21.2*	---	---	---	---	---	---	---	---	---	---	---
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 CO*CH$_3$*	---	---	21.2	---	21.2	21.2	21.1*	21.1	---	21.2	21.1	---	---	21.2	21.2	21.2	---	21.2

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Molecular formula of **6** is the same as that of **5** ($C_{31}H_{52}O_8$ *m/z* 552.3589 $[M]^+$). 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, $C_{33}H_{54}O_9$ (*m/z* 594.3768 $[M]^+$) 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 H_2-19.

Table 2. ^1H NMR (500 MHz) data for compounds **1-6** in $CDCl_3$

	1	**2**	**3**	**4**	**5**	**6**
Pos.	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	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),	β: 2.15 (dd, 3.4, 15.5),	β: 2.02 (m),	β: 2.03 (m),	β: 2.00 (m),	β: 2.60 (dd, 4.4, 15.8),
	α: 1.57 (m)	α: 1.55 (m)	α: 1.58 (m)	α: 1.59 (m)	α: 1.50 (m)	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), 1.57 (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),	β: 1.98 (m),	β: 1.99 (m),	β: 1.95 (m),	β: 1.97 (m),	β: 1.99 (m),
	α: 1.17 (m)	α: 1.18 (m)	α: 1.13 (m)	α: 1.25 (m)	α: 1.13 (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),	1.54 (m),	1.53 (m),	1.50 (m)	1.55 (m),	1.55 (m),
	1.03 (dd, 6.9, 11.7)	1.04 (m)	1.00 (m)	1.10 (m)	1.01 (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),	4.19 (d, 12.3),	4.17 (d, 12.6),	4.17 (d, 12.6),	4.16 (d, 12.6),	4.01 (d, 12.3),
	3.74 (d, 12.6)	3.76 (d, 12.3)	3.75 (d, 12.6)	3.75 (d, 12.6)	3.73 (d, 12.6)	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),	1.46 (m),	1.55 (m),	2.41 (dd, 6.3, 8.3)	1.66 (m),	1.68 (m),
	1.32 (m)	1.33 (m)	1.42 (m)	2.35 (dd, 6.3, 9.1)	1.47 (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), 139 (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)	0.88 (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 CO*CH*$_3$	---	2.07 (s)*	---	---	---	2.06 (s)
6 CO*CH*$_3$	2.05 (s)	2.06 (s)*	2.05 (s)	2.05 (s)	2.04 (s)	---
22 CO*CH*$_3$	---	---	2.02 (s)	---	2.02 (s)	2.03 (s)
OH						3.34 (br s)

*Interchangeable

Table 3. ^{1}H NMR (500 MHz) data for compounds **7-12** in $CDCl_3$

	7	**8**	**9**	**10**	**11**	**12**
Pos.	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),	1.97 (m),	1.56 (m),	1.54 (m),	1.55 (m),	2.05 (m),
	1.57 (m)	1.50 (m)	1.39 (m)	1.40 (m)	1.39 (m)	1.44 (m)
2	β: 1.71 (m),	1.81 (m),	β: 1.72 (m),	β: 1.71 (m),	β: 1.72 (m),	1.88 (m),
	α: 1.68 (m)	1.48 (m)	α: 1.55 (m)	α: 1.20 (m)	α: 1.23 (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),	β: 2.15 (dd, 4.1, 15.7),	β: 1.89 (m),	β: 1.90 (m),	β: 1.90 (m),	1.84 (m),
	α: 1.56 (m)	α: 1.56 (m)	α: 1.26 (m)	α: 1.54 (m)	α: 1.23 (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),	1.66 (m),	1.54 (m),	1.56 (m),	1.54 (m),	1.66 (m),
	1.46 (m)	1.52 (m)	1.51 (m)	1.51 (m)	1.51 (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),	1.53 (m),	1.39 (m),	1.37 (m),	1.38 (m),	1.55 (m),
	1.50 (m)	1.48 (m)	1.13 (m)	1.13 (m)	1.13 (m)	1.54 (m)
12	β: 1.96 (m),	β:1.98 (m),	β: 1.98 (ddd, 3.5, 3.5, 12.9),	β: 1.98 (m),	β: 1.98 (m),	β: 1.98 (m),
	α: 1.40 (m)	α: 1.13 (m)	α: 1.26 (m)	α: 1.20 (m)	α: 1.15 (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),	1.55 (m),	1.54 (m),	1.52 (m),	1.52 (m),	1.54 (m),
	1.01 (m)	1.01 (m)	1.10 (m)	1.08 (m)	1.08 (m)	1.04 (m)
16	1.91 (m),	1.89 (m),	1.88 (m),	1.90 (m),	1.90 (m),	1.89 (m),
	1.23 (m)	1.23 (m)	1.27 (m)	1.49 (m)	1.22 (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),	4.18 (d, 12.4),	3.82 (d, 8.5),	3.81 (d, 8.8),	3.81 (d, 8.5),	4.20 (d, 12.3),
	4.27 (d, 12.9)	3.75 (d, 12.4)	3.72 (d, 8.5)	3.72 (d, 8.8)	3.72 (d, 8.5)	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),	1.68 (m),	1.48 (m),	1.40 (m),	1.68 (m),	1.47 (m),
	1.48 (m)	1.50 (m)	1.35 (m)	1.55 (m)	1.51 (m)	1.33 (m)
24	1.40 (m),	1.67 (m),	1.26 (m),	1.09 (m),	1.40 (m),	1.26 (m),
	1.40 (m)	1.39 (m)	1.12 (m)	1.09 (m)	1.22 (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 CO*CH$_3$*	2.07 (s)	2.06 (s)	---	---	---	---
6 CO*CH$_3$*	---	2.05 (s)	---	---	---	2.07 (s)
19 CO*CH$_3$*	2.03 (s)*	---	---	---	---	---
22 CO*CH$_3$*	2.04 (s)*	2.02 (s)	---	2.02 (s)	2.03 (s)	
OH	3.40 (br s)					

*Interchangeable

The molecular formula $C_{33}H_{54}O_9$ (*m/z* 594.3769 $[M]^+$) 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 H_2-19 (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 $C_{27}H_{46}O_4$, 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 ^{1}H 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 $C_{29}H_{48}O_5$ (*m/z* 499.3386 [M + 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 $C_{29}H_{48}O_6$ (*m/z* 492.3455 [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.

Table 4. ^{1}H NMR (500 MHz) data for compounds **13-18** in $CDCl_3$

	13	**14**	**15**	**16**	**17**	**18**
Pos.	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), 1.55 (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) 2.19 (d, 13.2)
8	1.70 (m)	1.71 (m)	2.11 (m)	2.15 (m)	1.60 (m)	1.93 (m)
9	1.46 (m)	1.37 (m)	---	---	1.30 (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 CO*CH$_3$*	2.00 (s)	--	---	---	---	---
6 CO*CH$_3$*	2.07 (s)	2.06 (s)	2.06 (s)	---	---	---
22 CO*CH$_3$*	---	2.01 (s)	2.02 (s)	2.03 (s)	---	2.03 (s)

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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** $C_{29}H_{50}O_6$ (*m/z* 494.3627 $[M]^+$) 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 $C_{31}H_{52}O_8$ by HREIMS *m/z* 552.3663 $[M]^+$, 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×CH_3 (two from acetoxy groups), 11×CH_2 (one bonded to hydroxyl group), 8×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 $[M]^+$ *m/z* 494.3580 established the molecular formula $C_{29}H_{50}O_6$ 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×CH_3 (one from an acetoxy group), 10×CH_2, 8×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 $C_{29}H_{50}O_5$ (*m/z* 478.3641 $[M]^+$). The ^{13}C NMR and DEPT spectra of **17** (Table 1) showed the presence of 29 carbon signals assigned to 6×CH_3 (one from an acetoxy group), 10×CH_2, 9×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 $[M]^+$ which corresponds to the empirical formula $C_{29}H_{48}O_7$, 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×CH_3 (one from an acetoxy group), 9×CH_2, 8×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 the steroidal nucleus was established as all *trans* due to the observed NOEs between H_3-18 and H_3-19 (or H_2-19) 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).

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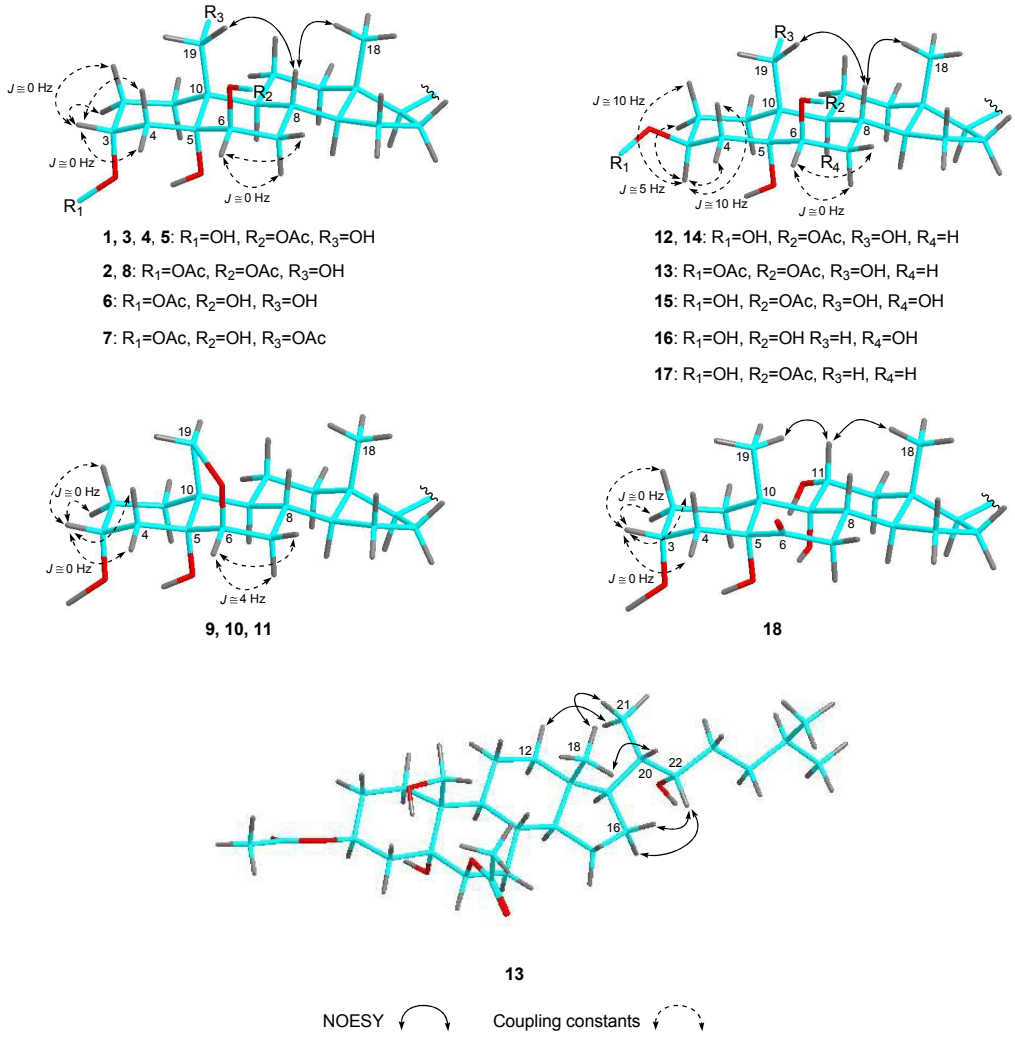

Fig. 2 Selected NOEs and coupling constants for compounds **1**–**18**.

Also, in **18** the NOEs observed between H-11 and H_3-18 and H_3-19 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 Δδ 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.

Table 5. ^{1}H NMR Δδ (δ_R–δ_S) values ($CDCl_3$, ppm, recorded at 500 MHz) of the MPA esters **14a** and **14b**

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

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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 H_3-18 and H-20 indicate a β disposition for the side chain. Also, NOEs observed between H-22 and H_2-16 and between H_3-21 and H-12β 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 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 3β-hydroxy-Δ^5 (ring A/B system) and side chains of eight carbon atoms, to give 3β,5α,6β–; 3β,5α,6β-19–oxy– and also isomerization to 3α,5α,6β–19–oxy–sterols derivatives. Δ^5 activation of the allylic position is an important way of providing polar steroids, 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.

Table 6. ^{1}H NMR $\Delta\delta$ (δ_R–δ_S) values ($CDCl_3$, 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

Antimicrobial activity

The antimicrobial activities of the steroids **1**, **3**, **4**, **6**, **8**–**10** and **13**–1**5** 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+ *B. cereus* and *Staphylococcus aureus* and Gram- *Klebsiella pneumoniae* with MIC values ranging from 25 to 50 µg/mL on the three strains. Compound **15** showed activity against *K. pneumoniae* with a MIC ≥ 50 µg/mL.

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 (≤ 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.

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

	% inhibition		
	Concentration (μM)		
Compd.	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

Toxicity for mammalian cells (primary mouse peritoneal macrophages) (Mφ) 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 Mφ viability by ca. 50% whereas the same concentration of **10** induced a mere 12% reduction in Mφ 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 (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 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.

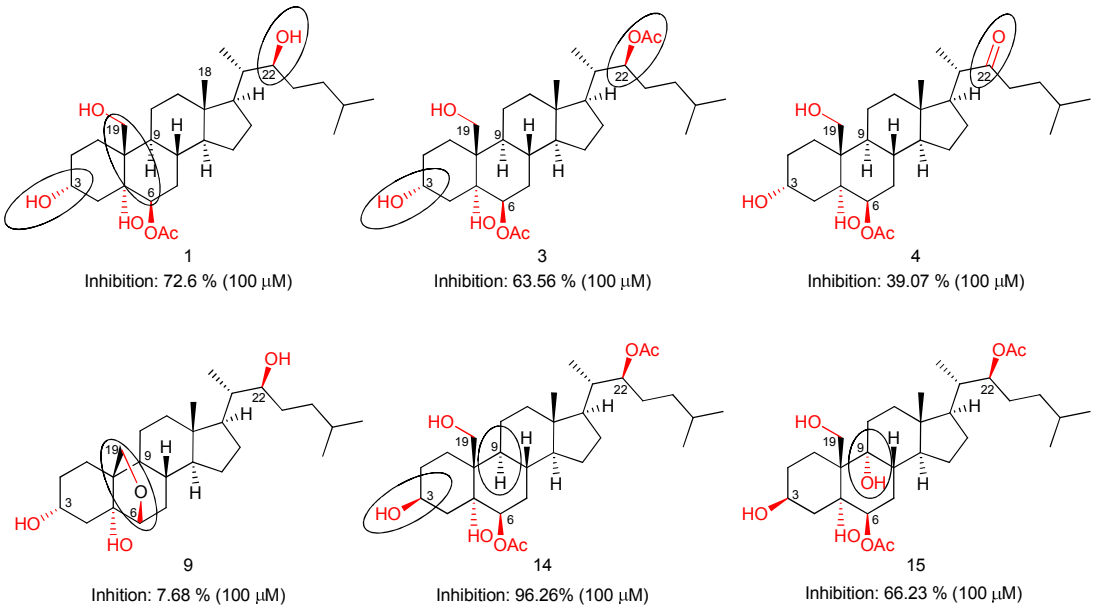

Fig. 3 Structure and antileishmanial effect of some of the isolated compounds.

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Experimental

General experimental procedures

Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 20 ºC. IR spectra were recorded on a Perkin-Elmer 1650/FTIR spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. All ^{13}C and ^{1}H 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 (posotive-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 semipreparative column (10μm, 20×250 mm) eluted with hexane/EtOAc mixtures. The spray reagent for TLC was H_2SO_4/H_2O/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 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; $[\alpha]^{20}_D$ − 49 (*c* 0.35, CH_2Cl_2); IR (film) ν_{max} 3420, 2944, 1716, 1258, 1031 cm^{-1}; EIMS *m/z* 494 $[M]^+$ (55), 476 $[M - H_2O]^+$ (75), 458 $[M - 2H_2O]^+$ (25), 440 $[M - 3H_2O]^+$ (13), 426 $[M - CH_3OH - 2H_2O]^+$ (22); HREIMS $[M]^+$ *m/z* 494.3622 (calcd for $C_{29}H_{50}O_6$, 494.3607), 476.3499 (calcd for $C_{29}H_{48}O_5$, 476.3502), 458.3409 (calcd for $C_{29}H_{46}O_4$, 458.3396), 440.3307 (calcd for $C_{29}H_{44}O_3$, 440.3290), 426.3134 (calcd for $C_{28}H_{42}O_3$, 426.3134); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 2. Isolated as an amorphous solid; $[\alpha]^{20}_D$ + 58 (*c* 0.12, CH_2Cl_2); IR (film) ν_{max} 3486, 2940, 1740, 1731, 1241, 1031 cm^{-1}; EIMS *m/z* 536 $[M]^+$ (14), 458 $[M - AcOH - H_2O]^+$ (58), 367 $[M–2AcOH - CH_2OH - H_2O]^+$ (14); HREIMS $[M]^+$ *m/z* 536.3731 (calcd for $C_{31}H_{52}O_7$, 536.3713), 458.3418 (calcd for $C_{29}H_{46}O_4$, 458.3396), 367.3002 (calcd for $C_{26}H_{39}O$, 367.3001); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 3. Isolated as an amorphous solid; $[\alpha]^{20}_D$ − 27 (*c* 0.46, CH_2Cl_2); IR (film) ν_{max} 3420, 2947, 1732, 1716, 1245, 1031 cm^{-1}; EIMS *m/z* 536 $[M]^+$ (10), 504 $[M - CH_3OH]^+$ (20), 458 $[M - AcOH - H_2O]^+$ (39), 440 $[M - AcOH\text{-}2H_2O]^+$ (27), 409 $[M - AcOH - CH_2OH - 2H_2O]^+$ (30), 398 $[M - 2AcOH - H_2O]^+$ (25), 380 $[M - 2AcOH - 2H_2O]^+$ (23), 367 $[M - 2AcOH - CH_2OH - H_2O]^+$ (15); HREIMS $[M]^+$ *m/z* 536.3688 (calcd for $C_{31}H_{52}O_7$, 536.3713), 504.3470 (calcd for $C_{30}H_{48}O_6$, 504.3451), 458.3386 (calcd for $C_{29}H_{46}O_4$, 458.3396), 440.3300 (calcd for $C_{29}H_{44}O_3$, 440.3290), 409.3123 (calcd for $C_{28}H_{41}O_2$, 409.3107), 398.3173 (calcd for $C_{27}H_{42}O_2$, 398.3185), 380.3064 (calcd for $C_{27}H_{40}O$, 380.3079), 367.3015 (calcd for $C_{26}H_{39}O$, 367.3001); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 4. Isolated as an amorphous solid; $[\alpha]^{20}_D$ + 59 (*c* 0.18, CH_2Cl_2); IR (film) ν_{max} 3420, 2940, 1732, 1716, 1241 cm^{-1}; EIMS *m/z* 492 $[M]^+$ (59), 460 $[M - CH_3OH]^+$ (17); HREIMS $[M]^+$ *m/z* 492.3434 (calcd for $C_{29}H_{48}O_6$ 492.3451), 460.3188 (calcd for $C_{28}H_{44}O_5$ 460.3189); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 5. Isolated as an amorphous solid; $[\alpha]^{20}_D$ − 34 (*c* 0.68, CH_2Cl_2); IR (film) ν_{max} 3412, 2944, 1731, 1714, 1246 cm^{-1}; EIMS *m/z* 552 $[M]^+$ (4), 521 $[M - CH_2OH]^+$ (9), 517 $[M - H_2O\text{-}OH]^+$ (8), 492 $[M - C_3H_8O]^+$ (54), 485 $[M - CH_2OH - 2H_2O]^+$ (10); HREIMS $[M]^+$ *m/z* 552.3671 (calcd for $C_{31}H_{52}O_8$, 552.3662), 521.3486 (calcd for $C_{30}H_{49}O_7$, 521.3478), 517.3537 (calcd for $C_{31}H_{49}O_6$, 517.3529), 492.3091 (calcd for $C_{28}H_{44}O_7$, 492.3087), 485.3281 (calcd for $C_{30}H_{45}O_5$, 485.3267); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 6. Isolated as an amorphous solid; $[\alpha]^{20}_D$ − 24 (*c* 0.28, CH_2Cl_2); IR (film) ν_{max} 3412, 2936, 1732, 1716, 1258 cm^{-1}; EIMS *m/z* 552 $[M]^+$ (9), 534 $[M - H_2O]^+$ (11), 516 $[M - 2H_2O]^+$ (8), 502 $[M - CH_3OH - H_2O]^+$ (10), 456 $[M - AcOH - 2H_2O]^+$ (80), 424 $[M - AcOH - CH_3OH - 2H_2O]^+$ (15), 414 $[M - 2AcOH - H_2O]^+$ (22); HREIMS $[M]^+$ *m/z* 552.3589 (calcd for $C_{31}H_{52}O_8$,

552.3662), 534.3571 (calcd for $C_{31}H_{50}O_7$, 534.3557), 516.3438 (calcd for $C_{31}H_{48}O_6$, 516.3451), 502.3311 (calcd for $C_{30}H_{46}O_6$, 502.3294), 456.3224 (calcd for $C_{29}H_{44}O_4$, 456.3240), 424.2980 (calcd for $C_{28}H_{40}O_3$, 424.2977), 414.3132 (calcd for $C_{27}H_{42}O_3$, 414.3134); ^{13}C and ^{1}H NMR see Tables 1 and 2.

Compound 7. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 173 (*c* 0.05, CH_2Cl_2); IR (film) ν_{max} 3442, 2940, 1729, 1714, 1376, 1246 cm^{-1}; EIMS *m/z* 594 $[M]^+$ (97), 456 $[M - 2AcOH - H_2O]^+$ (57), 346 $[M - C_3H_6O_2 - 2AcOH - 3H_2O]^+$ (10); HREIMS $[M]^+$ *m/z* 594.3768 (calcd for $C_{33}H_{54}O_9$, 594.3768), 456.3232 (calcd for $C_{29}H_{44}O_4$, 456.3240); 346.2674 (calcd for $C_{26}H_{34}$ 346.2661); ^{13}C and ^{1}H NMR see Table 1 and 3.

Compound 8. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 58 (*c* 1.16, CH_2Cl_2); IR (film) ν_{max} 3442, 2951, 1732, 1716, 1373, 1246 cm^{-1}; EIMS *m/z* 594 $[M]^+$ (20), 456 $[M - 2AcOH - H_2O]^+$ (20), 425 $[M - 2AcOH - CH_2OH - H_2O]^+$ (73); HREIMS $[M]^+$ *m/z* 594.3769 (calcd for $C_{33}H_{54}O_9$, 594.3768), 456.3235 (calcd for $C_{29}H_{44}O_4$, 456.3240), 425.3036 (calcd for $C_{28}H_{41}O_3$, 425.3056); ^{13}C and ^{1}H NMR see Table 1 and 3.

Compound 9. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 107 (*c* 0.18, CH_2Cl_2); IR (film) ν_{max} 3416, 2933, 1245, 1081 cm^{-1}; EIMS *m/z* 434 $[M]^+$ (7), 416 $[M - H_2O]^+$ (52), 398 $[M - 2H_2O]^+$ (79), 384 $[M - CH_3OH - H_2O]^+$ (2), 380 $[M - 3H_2O]^+$ (9), 366 $[M - CH_3OH - 2H_2O]^+$ (9), 348 [M- – CH_3OH – $3H_2O]^+$ (4); HREIMS $[M]^+$ *m/z* 434.3389 (calcd for $C_{27}H_{46}O_4$ 434.3396), 416.3291 (calcd for $C_{27}H_{44}O_3$, 416.3290), 398.3165 (calcd for $C_{27}H_{42}O_2$, 398.3185), 384.3047 (calcd for a $C_{26}H_{40}O_2$, 384.3028), 380.3097 (calcd for $C_{27}H_{40}O$, 380.3079), 366.2921 (calcd for $C_{26}H_{38}O$, 366.2923), 348.2805 (calcd for $C_{26}H_{36}$ 348.2817); ^{13}C and ^{1}H NMR see Table 1 and 3.

Compound 10. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 50 (*c* 0.14, CH_2Cl_2); IR (film) ν_{max} 3416, 2933, 1731, 1246, 1022 cm^{-1}; EIMS *m/z* 416 $[M - AcOH]^+$ (68), 349 $[M - AcOH - CH_2OH - 2H_2O]^+$ (20), 304 $[M - C_{10}H_{20}O_2]^+$ (93); HRESIMS $[M + Na]^+$ *m/z* 499.3386 (calcd for $C_{29}H_{48}O_5Na$ 499.3399); ^{13}C and ^{1}H NMR see Table 1 and 3.

Compound 11. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 300 (*c* 0.08, CH_2Cl_2); IR (film) ν_{max} 3408, 2933, 1731, 1248 cm^{-1}; EIMS *m/z* 492 $[M]^+$ (15), 474 $[M - H_2O]^+$ (7); HREIMS $[M]^+$ *m/z* 492.3455 (calcd for $C_{29}H_{48}O_6$, 492.3451), 447.3354 (calcd for $C_{29}H_{46}O_5$, 474.3345); ^{13}C and ^{1}H NMR see Table 1 and 3.

Compound 12. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 73 (*c* 0.35, CH_2Cl_2); IR (film) ν_{max} 3408, 2947, 1721, 1261, 1031 cm^{-1}; EIMS *m/z* 494 $[M]^+$ (91), 476 $[M - H_2O]^+$ (41), 458 $[M - 2H_2O]^+$ (56), 440 $[M - 3H_2O]^+$ (85), 385 $[M - AcOH - CH_2OH - H_2O]^+$ (7), 366 $[M - AcOH - CH_3OH - 2H_2O]^+$ (6); HREIMS $[M]^+$ *m/z* 494.3627 (calcd for $C_{29}H_{50}O_6$, 494.3607), 476.3487 (calcd for $C_{29}H_{48}O_5$, 476.3502), 458.3406 (calcd for $C_{29}H_{46}O_4$, 458.3396), 440.3275 (calcd for $C_{29}H_{44}O_3$, 440.3290), 385.3110 (calcd for $C_{26}H_{41}O_2$, 385.3107), 366.2918 (calcd for $C_{26}H_{38}O$, 366.2923); ^{13}C and ^{1}H NMR see Tables 1 and 3.

Compound 13. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 50 (*c* 0.34, CH_2Cl_2); IR (film) ν_{max} 3442, 2947, 1721, 1259, 1031 cm^{-1}; EIMS *m/z* 536 $[M]^+$ (4), 458 $[M - AcOH - H_2O]^+$ (8), 398 $[M - 2AcOH - H_2O]^+$ (2), 367 $[M - 2AcOH - CH_2OH - H_2O]^+$ (23); HREIMS $[M]^+$ *m/z* 536.3699 (calcd for $C_{31}H_{52}O_7$, 536.3713), 458.3406 (calcd for $C_{29}H_{46}O_4$, 458.3396), 398.3183 (calcd for $C_{27}H_{42}O_2$, 398.3185), 367.2985 (calcd for $C_{26}H_{39}O$, 367.3001); ^{13}C and ^{1}H NMR see Tables 1 and 4.

Compound 14. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 54 (*c* 0.63, CH_2Cl_2); IR (film) ν_{max} 3412, 2955, 1732, 1716, 1373, 1245, 1033 cm^{-1}; EIMS *m/z* 536 $[M]^+$ (2), 500 $[M - 2H_2O]^+$ (6), 458 $[M - AcOH - H_2O]^+$ (29); HRESMS $[M]^+$ *m/z* 536.3734 (calcd for $C_{31}H_{52}O_7$, 536.3713), 500.3505 (calcd for $C_{31}H_{48}O_5$, 500.3502), 458.3403 (calcd for $C_{29}H_{46}O_4$, 458.3396); ^{13}C and ^{1}H NMR see Tables 1 and 4.

Compound 15. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 91 (*c* 0.48, CH_2Cl_2); IR (film) ν_{max} 3394, 1732, 1718, 1245, 1035 cm^{-1}; EIMS *m/z* 552 $[M]^+$ (24), 534 $[M - H_2O]^+$ (17), 516 $[M - 2H_2O]^+$ (26), 502 $[M - CH_3OH - H_2O]^+$ (30); HREIMS $[M]^+$ *m/z* 552.3663 (calcd for $C_{31}H_{52}O_8$, 552.3662), 534.3582 (calcd for $C_{31}H_{50}O_7$, 534.3557), 516.3427 (calcd for $C_{31}H_{48}O_6$, 516.3451), 502.3291 (calcd for $C_{30}H_{46}O_6$, 502.3294); ^{13}C and ^{1}H NMR see Tables 1 and 4.

Compound 16. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 240 (*c* 0.04, CH_2Cl_2); IR (film) ν_{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 $C_{29}H_{50}O_6$, 494.3607), 477.3597 (calcd for $C_{29}H_{49}O_5$, 477.3580); ^{13}C and ^{1}H NMR see Tables 1 and 4.

Compound 17. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 263 (*c* 0.08, CH_2Cl_2); IR (film) ν_{max} 3531, 1716, 1243, 1072 cm^{-1}; EIMS *m/z* 478 $[M]^+$ (15), 400 $[M - AcOH - H_2O]^+$ (31); HREIMS $[M]^+$ *m/z* 478.3641 (calcd for $C_{29}H_{50}O_5$, 478.3658), 400.3342 (calcd for $C_{27}H_{44}O_2$, 400.3341); ^{13}C and ^{1}H NMR see Tables 1 and 4.

Compound 18. Isolated as an amorphous solid; $[\alpha]^{20}_D$ – 209 (*c* 0.14, CH_2Cl_2); IR (film) ν_{max} 3438, 2955, 1732, 1246 cm^{-1}; EIMS *m/z* 508 $[M]^+$ (18), 490 $[M - H_2O]^+$ (8); HREIMS $[M]^+$ *m/z* 508.3397 (calcd for $C_{29}H_{48}O_7$, 508.3400), 490.3300 (calcd for $C_{29}H_{46}O_6$, 490.3294); ^{13}C and ^{1}H NMR see Table 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 CH_2Cl_2 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 CH_2Cl_2 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 μg/ml) 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 penicillin + 100 μg/mL streptomycin + 1% L-glutamine and buffered with 20 mM HEPES at 26 ºC.[25] In the preliminary screening promastigotes (1.25 × 10^6 promastigotes /mL) were exposed to 10 μM, 50 μM and 100 μM of each steroid. To determine the approximate IC_{50} 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 ºC under CO_2/ air (5%/95%) atmosphere. *Leishmania* proliferation was determined by Alamar Blue method and the intensity of fluorescence (λ 550 excitation, λ 590 emission) measured. IC_{50} was calculated by least squares fit. Macrophage (Mφ) viability was determined using MTT method. Briefly, peritoneal Mφ were obtained from BAL/c mice and cultured (3.75 × 10^6 cells /mL) (RPMI medium) in 96 well flat-bottomed plates (Costar, Corning) in a final volume of 200 μM for 24 h under CO_2/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 MTT[26] and absorbance read at 570 nm. Experiments were done in triplicate.

Conclusions

During the course of our chemical investigation we identified eighteen new oxisterols. 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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Oxysterols from an octocoral of the genus *Gorgonia* from the eastern Pacific of Panama

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Eighteen new oxysterols were isolated from a previously undescribed octocoral of the genus *Gorgonia*. Antimicrobial and antileishmanial properties of these compounds have been evaluated.