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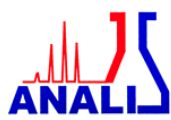
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## A-NOR STEROIDS FROM THE MARINE SPONGE, *Clathria* SPECIES

(Steroid A-nor daripada Span Laut, Spesies *Clathria*)

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### Abstract

Chemical compounds classified as A-nor steroids (**1–4**) were isolated from a marine sponge of the *Clathria* sp., collected from Southeast Sulawesi, Indonesia. Of these, one new compound, clathruhoate (**1**) was elucidated as 3 $\beta$ -(butyryloxymethyl)-A-nor-5 $\alpha$ -cholestane. All compounds were obtained using silica gel chromatography techniques and their structures were determined based on 1D and 2D NMR spectroscopic measurements and comparison with values from literature. Meanwhile, all compounds were inactive for antimicrobial activity.

**Keywords:** *Clathria* sp., marine sponge, A-nor steroid, 3 $\beta$ -(Butyryloxymethyl)-A-nor-5 $\alpha$ -cholestane

### Abstrak

Sebatian kimia diklasifikasikan sebagai steroid A-nor (**1–4**) telah diasingkan daripada span laut *Clathria* yang dikumpul dari Sulawesi Tenggara, Indonesia. Satu sebatian baharu, klathruhoat (**1**) telah ditentukan sebagai 3 $\beta$ -(butiriloksimetil)-A-nor-5 $\alpha$ -kolestan. Semua sebatian diperolehi dengan menggunakan teknik kromatografi gel silika dan struktur sebatian-sebatian ini telah ditentukan menggunakan spektroskopi 1D dan 2D NMR dan dengan perbandingan nilai dari kajian literatur. Sementara itu, semua sebatian didapati tidak aktif terhadap aktiviti antimikrob.

**Kata kunci:** *Clathria* sp., span laut, steroid A-nor, 3 $\beta$ -(Butiriloksimetil)-A-nor-5 $\alpha$ -kolestan

### Introduction

The diversity of marine species in the Indonesian Archipelago has been recognized worldwide [1, 2], and various species of sponges have been discovered in east Indonesia [3, 4]. The genus *Clathria* from the family Microcionidae is classified as Demospongia and contains approximately 544 accepted species worldwide [5]. Of these, some species from the marine regions of Australia, Argentina, Indo-Pacific, Korea, Panama, Philippines and New Zealand have been phytochemically investigated and found to contain alkaloids [6–12], amides [13], peptides [14, 15], anthraquinones [16], and terpenoids [17–23]. Meanwhile, biological activities including antibacterial [7, 11, 21, 22], anti-HIV [18], cytotoxicity towards cancer cell lines [9, 10, 13, 14, 22], anti-inflammatory [19], and antiplasmodial [24] have been attributed to the species in the genus *Clathria*.

In our extensive study on the diversity and chemistry of Indonesian marine sponges, we investigated the chemical constituents of *Clathria* sp. from the Southeast Sulawesi marine region. The investigation resulted in the isolation and elucidation of one new A-nor steroid (**1**), together with three known A-nor steroids (**2–4**). The isolated compounds (**1–4**) were also evaluated for their antimicrobial activity.

### Materials and Methods

#### Chemicals

Solvents used were distilled technical grades. Aluminium sheets kieselgel 60 PF<sub>254</sub> 0.25 mm (Merck 1.05554), silica gel 60 HF<sub>254</sub> 5-40  $\mu$ m (Merck 1.07747), and silica gel 60 GF<sub>254</sub> 5-40  $\mu$ m containing gypsum (Merck 1.07749) were used to perform thin layer chromatography (TLC), vacuum liquid chromatography (VLC), and radial chromatography (RC), respectively. Nutrient agar (NA), nutrient broth (NB), potato dextrose agar (PDA), potato dextrose broth (PDB), chloramphenicol, ketoconazole, and DMSO were used for antimicrobial assays.

#### Animal material

Samples of *Clathria* sp. (Figure 1) were collected by hand using SCUBA (Self Contained Underwater Breathing Apparatus) from the reef slope area (70°) of the Bintang Samudra Marine Education Park, Southeast Sulawesi, Indonesia, at a depth of 2–10 m, in May 2015. Samples (6.5 kg) were stored in ice and brought back shortly afterwards to the laboratory for further analysis. A sponge specimen was identified by the staffs of the Faculty of Fisheries and Marine Science of Universitas Halu Oleo with the registration number UHO-2015-03.



Figure 1. A photograph of the investigated *Clathria* sp.

#### Extraction and isolation

The dried powdered sponge (4 kg) was macerated with MeOH (3 x 8 L, 24 h each time) at room temperature and filtered. The solvent was evaporated under reduced pressure to yield the MeOH extract in the form of a brownish-yellow gum (300 g). A portion of this extract (30 g) was fractionated using silica gel vacuum liquid chromatography (VLC) (13 x 5 cm) with a gradient elution of *n*-hexane/EtOAc (9:1, 8:2, 7:3, 5:5, v/v) to produce five main fractions (F1-F5). Fraction F1 (2 g) was subjected to a silica gel radial chromatography (RC) and eluted isocratically with *n*-hexane/EtOAc (9.5:0.5, v/v) to yield nine subfractions (F1<sub>1</sub>-F1<sub>9</sub>). The combined subfractions F1<sub>1</sub>-F1<sub>3</sub> (1.36 g) were further separated on a silica gel RC with an elution of *n*-hexane/CHCl<sub>3</sub> (9.5:0.5, v/v) to afford compound **1** (30 mg). Purification of subfraction F1<sub>4</sub> (0.14 g) using a silica gel RC with *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> (6:4, v/v) as its mobile phase yielded compound **2** (23 mg). Fraction F2 (10 g) was subjected to a silica gel VLC (10 x 5 cm), eluted with *n*-hexane/EtOAc (9:1-0:10, v/v), and given eight subfractions (F2<sub>1</sub>-F2<sub>8</sub>). Subfraction F2<sub>4</sub> (0.8 g) was purified using silica gel RC with *n*-hexane/EtOAc/acetone (7:1:0.5, v/v) as its mobile phase to give compound **3** (200 mg). Purification of subfraction F2<sub>6</sub> (1.1 g) on a silica gel RC with the elution of *n*-hexane/EtOAc/acetone (7:1:0.5, v/v) yielded compound **4** (120 mg).

### Characterization study

The infrared (IR) analysis of compounds was performed using a Nicolet iS5 spectrophotometer with iD5 ATR (Thermo Scientific, Waltham, MA, USA). The HRESI-MS spectra were measured using a Waters QTOF mass spectrometer (Milford, MA, USA). Meanwhile, the  $^1\text{H}$ ,  $^{13}\text{C}$ , and 2D NMR spectra were recorded using a JEOL ECP 500 MHz (Tokyo, Japan) or an Agilent DD2 500 MHz (Agilent Technologies, Inc., Santa Clara, California, USA) spectrometers.

### In vitro antimicrobial assay

Antimicrobial assay for the isolated compounds was performed using an agar well diffusion method towards *E. coli* ATCC 35218, *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231 according to the previous method [30]. The development of microbial growth in a Petri dish was assisted using semisolid media. For bacterial growth, a semisolid medium containing sterilized nutrient agar (NA) (20 g/L) and nutrient broth (NB) (0.8 g/0.1 L) was prepared. Meanwhile, a semisolid medium for fungal growth was prepared with sterilized potato dextrose agar (PDA) (65 g/L) and potato dextrose broth (PDB) (2.4 g/0.1 L). One mL of microbial suspension (turbidity of 0.5 McFarland at 600 nm) was added into NB (5 mL) or PDB (5 mL) and mixed. Solutions of tested compounds were prepared with concentrations of 0.1, 0.2, and 0.3% in DMSO (10%, v/v). Chloramphenicol and ketoconazole each in 0.1% (v/v) were used as respective positive controls for bacteria and fungi, while DMSO was used as a negative control. The solid medium (10 mL) was poured into Petri dishes and allowed to solidify. Onto the solid medium, holes for samples were punched aseptically with tips. After that, the liquid medium-containing microbial suspension was evenly spread onto the surface of the solid medium. Samples (100  $\mu\text{L}$ ) were then added to their respective holes and the Petri dishes were incubated at 37 °C for 24 hours (bacteria) and 72 hours (fungi). Antimicrobial activity was evaluated by measuring the diameter of the clear zone around the hole. The inhibition zone of compounds and positive controls is categorized as strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm).

## Results and Discussion

### Characterization of isolated compounds

Purification of the methanol extract of the dried powdered *Clathria* sp. using silica gel vacuum liquid chromatography (VLC) and radial chromatography (RC) techniques yielded one new A-nor steroid, clathruoate (**1**) and other known A-nor steroids (**2–4**). Structures of these compounds are displayed in Figure 2.

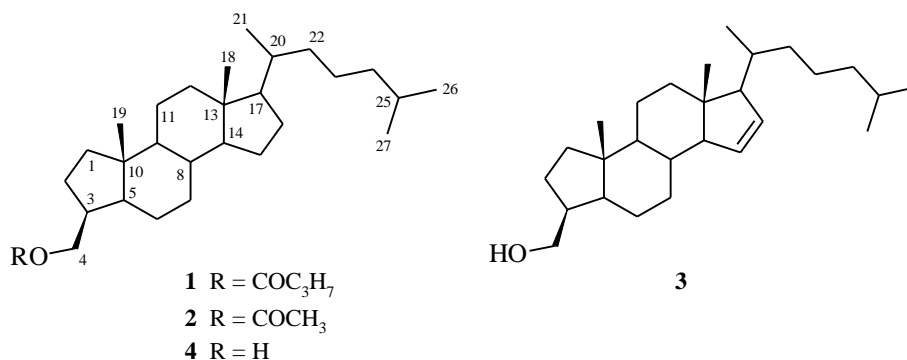


Figure 2. Chemical structures of compounds 1–4

By comparing the values between spectroscopic measurements and literatures data, compounds **2–4** were identified as  $3\beta$ -(acetoxymethyl)-A-nor-5 $\alpha$ -cholestane (**2**) [25],  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholest-15-ene (**3**) [25, 26], and  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholestane (**4**) [25]. A-nor sterols are produced by marine Demosponges (e.g. *Axinella* sp., *Homoaxinella* sp., *Phakellia* sp.) [25–28] via conversion of dietary sterols [29]. The occurrence of compounds **3** and **4** have been described in species *Axinella* [27, 28] and *Homoaxinella* [25], while the acetylation of compound **4** produced compound **2** [25]. Herein we report compound **2** as a natural product of the investigated *Clathria* sp. To our knowledge, the occurrence of these compounds in the genus *Clathria* is firstly reported in this work.

**3 $\beta$ -(butyryloxymethyl)-A-nor-5 $\alpha$ -cholestane (1)**

White solid. HRMS-ESI:  $m/z$  459.4112 [M+H]<sup>+</sup> (calculated for C<sub>31</sub>H<sub>55</sub>O<sub>2</sub><sup>+</sup>: 459.4197). ATR-FTIR ( $\nu_{\max}$ , cm<sup>-1</sup>): 2922, 2853, 1734, 1464, 1379, 1173, 968, 723. <sup>1</sup>H and <sup>13</sup>C NMR data are listed in Table 1.

Table 1. NMR data of compounds **1–4** (125 MHz for <sup>13</sup>C and 500 MHz for <sup>1</sup>H, in CDCl<sub>3</sub>,  $\delta$ , ppm, J/Hz)

C atom	1		2	3	4
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_C$	$\delta_C$
1	39.0	1.08 (1H, m), 1.61 (1H, m)	39.1	39.9	39.1
2	27.3	1.44 (1H, m), 1.94 (1H, m)	22.8	22.9	24.6
3	38.5	2.31 (1H, m)	38.6	39.7	42.9
4	68.3	3.94 (1H, dd, J=10.27, 6.85), 4.09 (1H, dd, J=10.27, 6.85)	68.5	67.2	66.7
5	52.4	1.44 (1H, m)	52.6	52.7	52.6
6	24.4	1.04 (1H, m), 1.55 (1H, m)	27.5	27.4	27.4
7	32.7	0.83 (1H, m), 1.74 m (1H, m)	32.8	32.9	32.8
8	35.8	1.36 (1H, m)	35.7	35.7	35.6
9	55.4	0.73 m (1H, m)	55.6	55.6	55.5
10	44.1	–	44.3	44.2	44.1
11	22.8	1.31 (2H, m)	23.4	24.6	22.8
12	39.5	1.14 (2H, m)	39.5	36.3	39.9
13	43.1	–	43.1	43.1	43.1
14	56.3	0.99 (1H, m)	56.4	56.5	56.5
15	23.8	1.14 (1H, m), 1.34 (1H, m)	24.6	130.0	24.6
16	29.3	1.26 (2H, m)	28.3	131.1	28.3
17	56.2	1.00 (1H, m)	56.4	56.4	56.4
18	12.2	0.66 (3H, s)	12.3	12.3	12.1
19	14.5	0.78 (3H, s)	14.6	14.7	14.4
20	35.5	1.29 (1H, m)	35.9	29.8	35.8
21	18.7	0.91 (3H, m)	18.9	18.9	18.7
22	36.2	0.99 (2H, m)	36.3	35.9	36.2
23	23.2	1.33 (1H, m), 1.44 (1H, m)	23.0	24.6	23.9
24	39.9	1.14 (1H, m), 1.95 (1H, m)	40.0	40.0	39.5
25	28.2	1.24 (1H, m), 1.81 (1H, m)	28.2	28.2	28.0
26	22.7	0.86 (3H, m)	22.7	22.7	22.7
27	22.6	0.86 (3H, m)	22.6	23.0	22.7
1'	174.1	–	171.5		
2'	34.5	2.27 (2H, t, J=7.83)	21.2		
3'	22.5	1.63 (2H, m)			
4'	14.1	0.89 (3H, m)			

Compound **1** was isolated as a white solid and was found to have a molecular formula of C<sub>31</sub>H<sub>55</sub>O<sub>2</sub> (MW 458.41238) based on a HRESI-MS analysis at  $m/z$  459.4112 [M + H]<sup>+</sup>, requiring five degrees of unsaturation. Of

these, one degree of unsaturation could be attributable to a carbonyl resonance ( $\delta$  174.1), while the remaining degrees were denoted by four rings. The IR spectrum showed absorption bands of methyl and methylene groups at  $2922\text{ cm}^{-1}$  ( $\text{CH}_3$  stretching),  $2853\text{ cm}^{-1}$  ( $\text{CH}_2$  stretching),  $1464\text{ cm}^{-1}$  and  $1379\text{ cm}^{-1}$  ( $\text{CH}_3$  and  $\text{CH}_2$  deformation) and an ester group at  $1734\text{ cm}^{-1}$  ( $\text{C}=\text{O}$  stretching) and  $1173\text{ cm}^{-1}$  ( $\text{C}-\text{O}$  stretching). The  $^1\text{H}$  NMR spectrum showed signals representing five resonances of methyl protons at  $\delta$  0.66 (H-18), 0.78 (H-19), 0.86 (H-26, H-27) and 0.91 (H-21) and two double doublet (dd) resonances of oxymethylene protons (H-4) at  $\delta$  3.94 ( $J=10.27, 6.85$ ) and 4.09 ( $J=10.27, 6.85$ ), indicating a typical  $3\beta$ -(hydroxymethyl)-*A*-nor cholestane [25, 26]. In addition, a methyl group at  $\delta$  0.89 (H-4') and two methylenes at  $\delta$  1.63 (H-3') and 2.27 (H-2') were observed in the spectrum, suggesting a butyryl group of an ester functionality in the structure as inferred in the IR analysis. In the  $^{13}\text{C}$  NMR spectrum, thirty-one carbon signals were observed, thus supporting the results from the mass analysis. The carbon of oxymethylene of the *A*-nor steroid was resonanced at  $\delta$  68.3 (C-4) and one carbonyl of ester functionality was resonanced at  $\delta$  174.1 (C-1'). The assignments of these protons and carbons in the skeleton of compound **1** were based on a HSQC analysis and are given in Table 1.

The presence of cholestane skeleton in compound **1** was verified using 2D NMR analyses, in particular, the HMBC measurement. Using this analysis, all carbons with the exception of C-2 ( $\delta$  27.3), C-6 ( $\delta$  24.4), C-11 ( $\delta$  22.8), and C-15 ( $\delta$  23.8) could be associated with one or more of the methyl groups based on  $^2J_{\text{CH}}$  and  $^3J_{\text{CH}}$  correlations (Figure 3). The patterns of these correlations were similar to the known compounds **2** and **4**, which confirmed **1** as an *A*-nor cholestane. The oxymethylene protons at C-4 and methine proton at C-3 showed  $^3J_{\text{CH}}$  and  $^4J_{\text{CH}}$  correlations to a carbonyl carbon ( $\delta$  174.1) of an ester functionality, respectively, confirming the position of this carbonyl at C-1'. The presence of a butyryloxy group ( $\text{C}_3\text{H}_7\text{COO}-$ ) was confirmed using the observed correlations of the methyl (H-4') and methylenes (H-2', H-3') protons toward the carbonyl carbon C-1'. In addition, protons H-2' also showed  $^4J_{\text{CH}}$  correlation to the oxymethylene carbon (C-4). Hence, this side group was attached to C-4. Based on these evidences, compound **1** was established as  $3\beta$ -(butyryloxymethyl)-*A*-nor-5 $\alpha$ -cholestane for which clathruhoate as a trivial name is proposed.

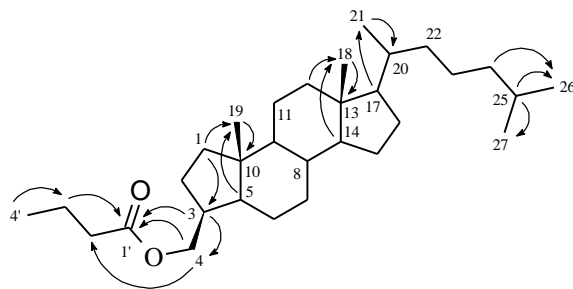


Figure 3. HMBC correlations (H $\rightarrow$ C) of compound **1**

### **3 $\beta$ -(acetoxymethyl)-*A*-nor-5 $\alpha$ -cholestane (2)**

Yellow crystal. MW: 430.7061 ( $\text{C}_{29}\text{H}_{50}\text{O}_2$ ). ATR-FTIR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 2926, 2858, 1740, 1455, 1370, 1164, 969, 721;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz,  $\delta/\text{ppm}$ , J/Hz): 4.09 (1H, dd,  $J=10.27, 7.15$ , H-4a), 3.95 (1H, t,  $J=8.4$ , H-4b), 2.33 (1H, m, H-3), 2.03 (3H, s, H-2'), 0.91 (3H, m, H-21), 0.86 (3H, m, H-27), 0.85 (3H, m, H-26), 0.75 (3H, s, H-19), 0.65 (3H, s, H-18).  $^{13}\text{C}$  NMR data are given in Table 1.

Compound **2** was obtained as a yellow crystal and gave a molecular formula of  $\text{C}_{29}\text{H}_{50}\text{O}_2$  (MW 430.7061) based on analyses using  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. According to this formula, five degrees of unsaturation were calculated as denoted by a carbonyl resonance ( $\delta$  171.5) and four rings of the cholestane skeleton. The IR spectrum of compound **2** showed the presence of methyl and methylene groups ( $2926, 2858, 1455, 1370\text{ cm}^{-1}$ ) and an ester group ( $1740, 1164\text{ cm}^{-1}$ ), which was similar to compound **1**. The  $^1\text{H}$  NMR spectrum exhibited six resonances of methyl protons at  $\delta$  0.65 (s, H-18), 0.75 (s, H-19), 0.85 (m, H-26), 0.86 (m, H-27), 0.91 (m, H-21) and 2.03 (s, H-2') and multiplet resonances of typical cholestane protons ranging from  $\delta$  0.83 to 2.33. The oxymethylene protons (H-4) were

observed as a double doublet at  $\delta$  4.09 (J=10.27, 7.15, H-4a) and a triplet at  $\delta$  3.95 (J=8.14, H-4b). Meanwhile, the  $^{13}\text{C}$  NMR spectra showed twenty-nine carbons in the skeleton of compound **2** (Table 1), and their values were identical to those given in [25]. Hence, compound **2** was deduced as  $3\beta$ -(acetoxymethyl)-A-nor-5 $\alpha$ -cholestane.

### **$3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholest-15-ene (3)**

White solid. MW: 386.6535 ( $\text{C}_{27}\text{H}_{46}\text{O}$ ). ATR-FTIR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3334, 2918, 2853, 1594, 1508, 1454, 1376, 1265, 1035, 977.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz,  $\delta/\text{ppm}$ , J/Hz): 5.15 (1H, m, H-15), 5.13 (1H, m, H-16), 3.71 (1H, dd, J=10.45, 6.50, H-4a), 3.47 (1H, t, J=9.72, H-4b), 2.22 (1H, m, H-3), 1.95 (2H, m), 1.82 (1H, m), 0.90 (3H, m, H-21), 0.89 (3H, m, H-27), 0.85 (3H, m, H-26), 0.74 (3H, s, H-19), 0.65 (3H, s, H-18).  $^{13}\text{C}$  NMR data are given in Table 1.

Compound **3** was obtained as a white solid. The compound has a molecular formula of  $\text{C}_{27}\text{H}_{46}\text{O}$  (MW 386.6535) based on  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral analyses and required five degrees of unsaturation, originating from one olefinic group ( $\delta$  130.0, 131.1) and four rings of the cholestane structure. The IR spectrum displayed absorption bands of a hydroxyl group ( $3334\text{ cm}^{-1}$ , OH stretching), methyl and methylenes groups ( $2918, 2853, 1454, 1376\text{ cm}^{-1}$ ), and an alkene group ( $1594\text{ cm}^{-1}$ , C=C stretching). The  $^1\text{H}$  NMR spectrum of compound **3** exhibited resonances of olefinic protons at  $\delta$  5.15 (m, H-15) and 5.13 (m, H-16), oxymethylene protons at  $\delta$  3.71 (dd, J=10.45, 6.50, H-4a) and 3.47 (t, J=9.72, H-4b), and methyl protons at  $\delta$  0.65 (s, H-18), 0.74 (s, H-19), 0.85 (m, H-26), 0.89 (m, H-27) and 0.90 (m, H-21). The presence of a hydroxyl group in the IR spectrum and oxymethylene protons ( $\delta$  3.71, 3.47) in the  $^1\text{H}$  NMR spectrum suggested an alcohol functionality in compound **3**. The  $^{13}\text{C}$  NMR spectrum consisted of twenty-seven carbons including two olefinic carbons ( $\delta$  130.0, 131.1) and one oxymethylene carbon ( $\delta$  67.2) (Table 1). The NMR data of compound **3** were identical with those assigned for  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholest-15-ene [25, 26]. This compound was previously reported from *Homoaxinella trachys* [25], *Phakellia aruensis* [26], and *Axinella proliferans* [27].

### **$3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholestane (4)**

White solid. MW: 388.6694 ( $\text{C}_{27}\text{H}_{48}\text{O}_2$ ). ATR-IR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3357, 2915, 2820, 1454, 1378, 1276, 1196, 740;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz,  $\delta/\text{ppm}$ , J/Hz): 3.70 (1H, dd, J=10.3, 6.3, H-4a), 3.46 (1H, t, J=9.6, H-4b), 2.23 (1H, m, H-3), 1.93 (2H, m), 1.82 (1H, m), 0.91 (3H, m, H-21), 0.86 (3H, m, H-27), 0.84 (3H, m, H-26), 0.73 (3H, s, H-19), 0.64 (3H, s, H-18).  $^{13}\text{C}$  NMR data are given in Table 1.

Compound **4** was isolated as a white solid and have a molecular formula of  $\text{C}_{27}\text{H}_{48}\text{O}_2$  (MW 388.6694). Four degrees of unsaturation calculated from this formula was attributed by four rings of the cholestane structure. The IR spectrum suggested a hydroxyl group ( $3357\text{ cm}^{-1}$ ) and methyl and methylenes groups ( $2918, 2853, 1454, 1376\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum showed two coupled peaks at  $\delta$  3.70 (dd, J=10.3, 6.3) and 3.46 (t, J=9.6), indicating the mutual oxymethylene protons as previously observed in compounds **1–3**. Similarly, five methyl groups of typical cholestane skeleton were observed at  $\delta$  0.64 (s, H-18), 0.73 (s, H-19), 0.84 (m, H-26), 0.86 (m, H-27) and 0.91 (m, H-21). Meanwhile, as many as twenty-seven carbons were obtained from the  $^{13}\text{C}$  NMR spectrum and their values were identical to compounds **1–2** with the exception of butyryloxy and acetoxy carbons. Hence, compound **4** was determined as  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholestane [25]. This compound was previously reported from *Axinella verrucosa* [28].

### **Antimicrobial activity of isolated compounds**

The isolated compounds were evaluated for antibacterial activity against *E. coli*, *S. aureus*, and antifungal activity against *C. albicans*. However, none of the tested compounds exhibited antibacterial and antifungal activities as compared to chloramphenicol and ketoconazole, respectively. To our knowledge, the antimicrobial activity of these compounds is reported for the first time in this study.

### **Conclusion**

Four A-nor steroids were isolated and identified from the methanol extract of the marine sponge *Clathria* sp. in the genus *Clathria* for the first time, which are  $3\beta$ -(butyryloxymethyl)-A-nor-5 $\alpha$ -cholestane (**1**),  $3\beta$ -(acetoxymethyl)-A-nor-5 $\alpha$ -cholestane (**2**),  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholest-15-ene (**3**), and  $3\beta$ -(hydroxymethyl)-A-nor-5 $\alpha$ -cholestane (**4**). Compound **1** named as clathruhoate was established as a new compound from this species. The

presence of these compounds could enrich the chemical diversity of the genus *Clathria*. Despite their inactive properties towards *E. coli*, *S. aureus*, and *C. albicans*, further studies are still required to explore more about the potent antimicrobial compounds from this species as well as other biological activities.

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