

## New polyhydroxy sterols from the marine sponge *Callyspongia fibrosa* (Ridley & Dendly)

Thota S. Prakasa Rao<sup>a</sup>, Nittala S. Sarma\*<sup>a</sup>, Y.L.N. Murthy<sup>a</sup>,  
Venkata S.S.N. Kantamreddi<sup>b</sup>, Colin W. Wright<sup>b</sup>, P.S. Parameswaran<sup>c</sup>

<sup>a</sup>*School of Chemistry, Andhra University, Visakhapatnam – 530 003, India*

<sup>b</sup>*School of Pharmacy, University of Bradford, Bradford-BD7 1DP, U.K.*

<sup>c</sup>*National Institute of Oceanography, Dona Paula, Goa-403 004, India*

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**Abstract** - Four new polyhydroxylated sterols are isolated from Marine sponge *Callyspongia fibrosa* collected from the Gulf of Mannar, western Bay of Bengal (India). The structural assignment is based on <sup>1</sup>H and <sup>13</sup>C NMR spectra. All sterols are based on the known 24*S*-24-methyl cholesterol **1** which is also isolated, and contain 3β,6β-dihydroxy system and 25-*O*- acetate as common features (except in the case of sterol **6** that has a Δ<sup>25</sup> in the place of 25-OAc). Additional OH substitution is also present at 5α in **4a** and at 8β in **5**. A further 12β-OH is present in **6** and **7**. The hydroxylation pattern is so far known only in coral sterols but is without a precedent in sponge sterols. The major steroid **4a** showed antimalarial activity against *Plasmodium falciparum* on the chloroquine resistant stain better than on the chloroquine sensitive strain.

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Marine sponges retain their importance as one of the choicest class of organisms for isolating new and novel biologically active molecules despite several new classes of organisms like bacteria, tunicates, microalgae, bryozoans etc. offering increased promise in recent years. For polyhydroxy sterols, marine sponges enjoy a distinct place<sup>1</sup> as they are likely candidates for drug development, e.g., a derivative of contignasterol from *Petrosia contignata*<sup>2</sup> which is in Phase II clinical trials as antiasthmatic.<sup>3</sup> In many sponges, polar sterols occur as major constituents. Hence, any physiological activity that can be associated with these major principles of sponges would be practically beneficial.

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**Keywords:** *Callyspongia fibrosa*; polyhydroxylated steroid; antimalarial activity

\*Corresponding author. E-mail: nssarma@rediffmail.com

It was with this objective that we have continued our interest on marine sponge sterols. *Callyspongia fibrosa* is a sponge occurring in tropical waters. To our knowledge, only one publication is available reporting a pyridinium alkaloid from a Micronesian (Polynesia in the tropical Pacific Ocean) collection.<sup>4</sup> From species of *Callyspongia* other than *C. fibrosa*, in addition to pyridinium salts, polyacetylenic compounds,<sup>5</sup> and more recently depsipeptides<sup>6</sup> and hexaprenoid derivatives<sup>7</sup> that are associated with biological activity have been reported in literature. But so far, no report of sterols or their polyhydroxy derivatives is available from any species of *Callyspongia*. Our examination of *C. fibrosa* collected from the Gulf of Mannar (Bay of Bengal) yielded seven compounds of which six are steroids: two known namely 24S-24-methylcholesterol (**1**), and 24S-24 methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetraol-25-mono acetate (**4a**) and four **3-7** new. The seventh compound is the known batyl alcohol (**2**), which is commonly encountered in marine invertebrates. The structural elucidation of the new steroids is described in the paper. We have also conducted testing of biological activity of the seven isolated compounds against malarial parasite, and found significant activity for the known sterol **4a**, the details of which are also presented.

The sponge (voucher specimen: AU1-274) was collected from Mandapam coast in the Gulf of Mannar of the Indian Ocean during April 2003. After cleaning, it was soaked in methanol and later the squeezed-out material (dry mass: 1.5 kg) extracted with CH<sub>3</sub>OH at room temperature. The EtOAc soluble portion of the extract, a dark greenish gummy residue (40g) was subjected to column chromatography on silica gel (Acme, 100-200 mesh, 250g) by gradient elution with *n*-hexane (100-70%) in ethyl acetate. Fractions 250ml each were monitored by TLC. Compounds **1** (yield: 10 mg), **2** (25) and **7** (25) were obtained by repeated crystallization of pooled fractions directly. Compounds **3** (10 mg) & **4a** (65)

and **5** (35 mg) & **6** (40) were also obtained from pooled fractions but after rechromatography. The antimalarial activity was carried out at Bradford school of pharmacy, University of Bradford, UK. Cultures containing predominantly early ring stages of *Plasmodium falciparum* were used for testing. Chloroquine diphosphate was used as positive control and uninfected and infected erythrocytes without sample solutions were incubated in each test plates were placed into a modular incubator gassed with 93% N<sub>2</sub>, 3% O<sub>2</sub>, and 4% CO<sub>2</sub> and incubated at 37 °C for 48hr. Parasite growth was assessed as activity (PLDH) as described.<sup>8</sup> The reagent used contained the following in each micro-litre: acetylpyridine adenine dinucleotide (APAD, 0.74 mg; lithium lactate, 19.2 mg; diaphorase, 0.1 mg; Triton X-100, 2µl; nitroblue tetrazolium, 1mg; and phenazine ethosulfate, 0.5mg. Fifty microlitres of this reagent was added to each well and mixed, and plates were read at 550nm and percent inhibition of growth was calculated by comparison with control values. The IC<sub>50</sub> values were determined using linear regression analysis.<sup>9,10</sup> A minimum of three separate determinations was carried out for each sample.

Compound **1**, colorless needles from chloroform-methanol, M<sup>+</sup> 400 for C<sub>28</sub>H<sub>48</sub>O (EI) was established as 24*S*-24-methylcholesterol (**1**) by comparison of <sup>1</sup>H and <sup>13</sup>C NMR and Mass spectra previously reported for this compound isolated from a number of soft corals e.g., *Sinularia*<sup>11</sup> and *Lobophytum*<sup>12</sup> genera. Final confirmation came from comparison with an authentic sample (mmp and superimposable IR spectra).<sup>13</sup> Compound **4a**, colorless needles from chloroform-methanol; C<sub>30</sub>H<sub>52</sub>O<sub>5</sub> is major polar sterol of the sponge. It is a naturally occurring *O*-acetate that gave triacetate on py-Ac<sub>2</sub>O (RT) reaction. Its physical and spectral characteristics agreed with the known sterol 24*S*-24-methyl cholestane-3β,5α,6β,25-tetraol-25-*O*-acetate isolated first from several soft coral species e.g., *Sarcophyton elegans*<sup>14</sup> and several other species subsequently including the gorgonian

*Junceella juncea*.<sup>15</sup> The spectral characteristics agreed with those presented recently on the sample isolated from *Lobophytum sp.* of the Gulf of Mannar, India.<sup>13</sup> The triacetate **4b** prepared easily by Ac<sub>2</sub>O/py reaction of **4a** also is in accord for 24*S*-24-methylcholestane-3β,5α,6β,25-tetraol-3,6,25-*O*-triacetate.<sup>16</sup> Final confirmation of **4a** came from mmp and superimposable IR spectra and by direct comparison with an authentic sample.<sup>17</sup> To our knowledge, the report of **4a** from a sponge is a first time record.

Compound **3**, colourless needles from CHCl<sub>3</sub> (with traces of CH<sub>3</sub>OH) is C<sub>30</sub>H<sub>50</sub>O<sub>3</sub> derived from the combination of elemental analysis and molecular ion (M<sup>+</sup>) 458 in the EIMS. IR showed absorption at 3433-3358cm<sup>-1</sup> for hydroxyl groups, and 1728cm<sup>-1</sup> for carbonyl group (acetate). The compound gave LB test for sterols with characteristic play of colors (pink-blue-green). Compound **3** has seven methyl groups including acetoxy group whose protons appear as sharp singlet at δ 1.94 in the <sup>1</sup>H NMR. Of the remaining six, two are secondary (δ0.91d J=5.4Hz, 21-CH<sub>3</sub> and δ0.89d J=5.4Hz, 28-CH<sub>3</sub>) and remaining four tertiary. Two of the tertiary methyls are at down field, i.e., δ1.38 and 1.40 being geminal to the acetoxy group (C-25) in the side chain, as in the case of **4a**. The other two tertiary methyls are at 1.14s and 0.70s as for **4a**. The configuration at C-24 in this and congener sterols is suggested as 24*S* by analogy with other sterols, and from the observation that the 24*S* (α)-methyl protons appear downfield by 0.02 ppm compared to 24*R* (β) protons, and that with 24*S*, the C-21 doublet experiences shielding by as much as 0.09 ppm.<sup>18</sup> As for protons adjacent to the OH groups, compound **3** contains signals at 4.02m (1H) for 3α-H and 3.44brs (1H) for 6α-H, positions similar to those in **4a** at 3.98m (1H) and 3.42brs (1H) respectively. The 6β-OH assignment is in line with other sterols containing this group, very common amongst coral sterols<sup>19</sup>. The difference between **3** and **4a** is shown in <sup>13</sup>C NMR. Sterol **3** contains only three oxycarbons instead of four; A new signal appears at δ 40.1 in

the place the tertiary oxycarbon (from DEPT) at 75.1. The absence of 5 $\alpha$ -OH in **3** causes significant shielding of  $\beta$ -carbons 4, 6 and 10 and minor deshielding of  $\gamma$ -carbons 7 and 19<sup>20</sup> with respect to **4a**. Hence, the structure of **3** is established as 24*S*-24-methyl cholestane 3 $\beta$ ,6 $\beta$ ,25-triol-25-*O*-acetate. In marine sponges, the 5 $\alpha$ (H),6 $\beta$ -hydroxy system has not been so far reported, but the isomeric 5 $\alpha$ (H),6 $\alpha$ -hydroxy system has been reported<sup>1</sup> in sterols that also contain  $\Delta^7$  or  $\Delta^{8(14)}$ . In soft corals, a few sterols containing 5 $\alpha$ (H), 6 $\beta$ -hydroxy system were reported; thus compound **3** is established as 24*S*-24-methyl cholestane -3 $\beta$ ,6 $\beta$ ,25-triol -25-*O*-acetate is indeed novel.<sup>19</sup>

Compound **5**, C<sub>30</sub>H<sub>52</sub>O<sub>5</sub> (by elemental analysis and at M<sup>+</sup> 492), is also a polyhydroxy sterol containing one methyl carboxylate group from its IR absorption bands at 3423-3258cm<sup>-1</sup> (O-H stretching), and 1739cm<sup>-1</sup> (C=O stretching of acetate) and the corresponding <sup>1</sup>H NMR singlet at  $\delta$ 1.95 for the methyl protons. As in the case of **3** and **4a**, the <sup>1</sup>H NMR signals of the C-26 and C-27 methyl protons appear at  $\delta$ 1.38s and 1.40s that justify 25-OAc substituent. Three additional oxy carbons, apart from this carbon were shown in the <sup>13</sup>C NMR, two secondary 64.01 (3-C) and 75.48 (6-C) and one tertiary 75.20 (8-C). The 18 and 19-methyl proton signals of **5** are deshielded by 0.01 ppm with respect to **3** and **4a** respectively, and this deshielding, however small, can be caused by 8 $\beta$ -OH which is 1,3-syndiaxial to both 18-Me and 19-Me groups. Alternative locations that could have been considered for the extra tertiary -OH group were C-9 and C-14 but since the influence of each of them can be confined only to one position (19-H<sub>3</sub> and 18-H<sub>3</sub> respectively) and not both, these positions were not favoured. Sterols containing 8 $\beta$ -OH group are rare. Recently, stylisterols A and B that contain this group are reported from the marine sponge *Stylissa* sp. from Okinawa, Japan<sup>21</sup> with which the methyls assignment agrees. Thus **5** is 24*S*-24-methyl cholestane-3 $\beta$ ,6 $\beta$ ,8 $\beta$ ,25- tetraol-25-*O*-acetate.

Compound **6** analysed for the empirical formula  $C_7H_{12}O$  that together with  $M^+$  of 444 suggested the molecular formula as  $C_{28}H_{48}O_4$  and the presence of five degrees of unsaturation in the molecule. It was transparent to UV-Visible light  $>220$  nm but showed absorptions in the IR region at  $3490-3304\text{ cm}^{-1}$  that pointed to the presence of hydroxyl groups. The first indication that the compound could be a sterol came from LB test with characteristic play of colors (pink-blue-green), and later from the  $^1H$  NMR spectrum that showed signals for five methyl groups. A deviation from the usual sterol methyls is that instead of six methyl groups for the sterol C-9 side chain, **6** has only five methyl groups. A new vinylic group appears at ( $\delta$  4.65s, 2H) that causes 0.2 ppm deshielding of 27-Me (to 1.62 ppm). In the  $^{13}C$  NMR signals at 108.89 (26-C) and 149.69 (25-C) in the place of oxycarbon ( $\delta$ 86.06) and methyl carbon (24.01) of compound **5**. Thus the new vinylic methylene is located at C-25. The  $^{13}C$  NMR ( $CD_3OD$ ) spectrum showed the presence of four oxygenated carbon atoms, one tertiary and three secondary in the DEPT mode. Of the three secondary OH groups, one is the ubiquitous 3-OH ( $\delta$ 4.0dd). The other two are at C-6 $\beta$  (3.42brs) and C-12 $\beta$  (4.00dd). The C-12 $\beta$ -OH is suggested by the significant shielding experienced by methyl carbons, the nearby 19-C ( $\delta$ 11.26) and the remote 18-C (8.84).<sup>22,23</sup> In the proton COSY and DQF COSY spectra, 12 $\alpha_{ax}$ -H shows a good connectivity with 21s-CH<sub>3</sub> in **6**. Thus, compound **6** is 24*S*-24-methyl cholest-25-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,12 $\beta$ -tetrol, a new compound to literature.

The molecular formula of **7** is  $C_{30}H_{52}O_6$  derived from a combination of elemental analysis and Mass spectra. An indication that this compound is also a polyhydroxy steroid came from LB test and  $^1H$  NMR spectra. In the IR, an absorption band, over and above the group of signals at  $3400-3450\text{ cm}^{-1}$  for OH group is in the carbonyl region at  $1724\text{ cm}^{-1}$  (ester). Its corresponding acetoxy ( $CH_3COO-$ ) protons appear as sharp singlet at  $\delta$ 1.96 in

the  $^1\text{H}$  NMR. The acetoxy group is attached to the isopropyl methine carbon in the side chain as in the case of compounds **3-5**. Thus, two singlets appear at the deshielded positions of 1.38 and 1.40. There are two secondary methyls, one at 0.93d J=5.4 Hz for the ubiquitous 21-CH<sub>3</sub> group and another at 0.89d J=5.4 Hz for 28-CH<sub>3</sub>, as for compound **6**. In the downfield, the multiplet signal at 3.94m integrating for two protons, 3-H and 12-H and a relatively upper field signal of 3.41brs for 6-H are as previously assigned. Thus **7** is 24S-24-methyl chelestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,12 $\beta$ ,25-pentaol-25-O-acetate.

Of the seven tested compounds, three (**1**, **2** and **5**) were inactive. The four remaining viz., compounds **3**, **4a**, **6** and **7** exhibited moderate activity (Table 3: Supplementary Section) against *P. falciparum*. Compound **4a** yields a relatively better result. Interestingly, this compound, contrary to chloroquine, shows a better activity against chloroquine resistant strain than the chloroquine sensitive strain of *P. falciparum*.

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### References

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24 Spectral data of a few selected compounds:

Compound **1**: Colourless needles (MeOH:CHCl<sub>3</sub>=1:3), m.p. 174-176°C,  $[\alpha]_{\text{D}}^{25} -35^{\circ}$  (c, 1.0 CHCl<sub>3</sub>); analyzed for C<sub>28</sub>H<sub>48</sub>O (calcd. C 84.0%, H 12.0%; found: 83.8% and 12.1% respectively); FTIR (CHCl<sub>3</sub>): 3632, 2953, 1458, 1386, 1034cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ values 3.50 (brs, m 3β-H), 0.90 (d, 6hz, 28-H), 5.34 (d), 0.97 (s), 0.80 (d, 6Hz, 26-H), 0.83 (d, 6Hz, 27-H), 0.94 (d, 6Hz, 21-H), 0.66 (s), EIMS: m/z 400 [M<sup>+</sup>], 382.

Compound **2**: Colourless needles (MeOH), m.p. 69-71 °C,  $[\alpha]_{\text{D}}^{25} +2.9^{\circ}$  (c 1.0, CHCl<sub>3</sub>); analyzed for C<sub>21</sub>H<sub>44</sub>O<sub>3</sub> (calcd. C 73.2, H 12.7%; found: 73.1% and 12.8% respectively); FTIR (CHCl<sub>3</sub>): 3454, 2924, 1456, 1125, 1045, 870cm<sup>-1</sup>; <sup>1</sup>H NMR (CHCl<sub>3</sub>) 3.86 (1H, q 6.5Hz); 3.67-3.70 (2H, m); 3.43-3.58 (4H, m); 2.6 (1H, d, 6.5Hz); 2.15 (1H, 6.5Hz); 1.60 (2H, m); 1.28 (28H, brs); 0.88 (3H, t, 6.5Hz); EIMS [M<sup>+</sup>] 344, 313.

Compound **3**: Colourless needles (MeOH:traces of CHCl<sub>3</sub>), m.p. 180-182°C,  $[\alpha]_{\text{D}}^{25} -29^{\circ}$  (c, 1.03 CH<sub>3</sub>OH); analyzed for C<sub>30</sub>H<sub>50</sub>O<sub>4</sub> (calcd. C 75.6%, H 12.9%; found: 75.4% and 11.0% respectively); FTIR (CD<sub>3</sub>OD): 3433, 3358, 2914, 2893, 2870, 2848, 1728, 1359, 1253, 1045, 932cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2 in supplementary section; EIMS [M<sup>+</sup>] 476.

Compound **4a**: Colourless needles (MeOH: CHCl<sub>3</sub>=3:1) m.240-242°C  $[\alpha]_{\text{D}}^{25} -19.9^{\circ}$

(c 1.5, MeOH); analyzed for C<sub>30</sub>H<sub>52</sub>O<sub>5</sub> (calcd. C 73.1%, H 10.5%; found: 72.9% and 10.7% respectively); FTIR (CD<sub>3</sub>OD): 3512, 2992, 1732, 1418, 1262, 935cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 1 and 2 in supplementary section; EIMS [M<sup>+</sup>] 432, M<sup>+</sup>-AcOH, 414, 381, 305, 287, 109, 55.

Compound **4b**: M.p. 175-177°C, [α]<sub>D</sub><sup>25</sup>-48.7° (c 0.97, CH<sub>3</sub>OH), analyzed for C<sub>34</sub>H<sub>56</sub>O<sub>7</sub> (calcd. C 84.0%, H 12.0%; found: 83.8% and 12.1% respectively); FTIR (CHCl<sub>3</sub>): 3460, 2953, 2852, 1749, 1242, 1032, 961cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) 0.69 (3H, s); 0.93 (3H, d, 6.6Hz); 0.87 (3H, d, 6.9Hz); 1.40 (3H, s); 1.40 (3H, s); 1.17 (3H, s); 4.70 (1H, brs, s); 5.15 (1H, m); 1.98 (3H, s), 2.02 (3H, s); 2.05 (3H, s); <sup>13</sup>C NMR 31.7 (C-1), 26.6 (C-2), 70.5 (C-3), 36.8 (C-4), 74.9 (C-5), 76.1 (C-6), 31.3 (C-7), 30.6 (C-8), 55.7 (C-9), 38.4 (C-10), 21.0 (C-11), 39.8 (C-12), 42.6 (C-13), 55.8 (C-14), 24.0 (C-15), 28.0 (C-16), 45.0 (C-17), 12.1 (C-18), 16.3 (C-19), 36.2 (C-20), 18.9 (C-21), 34.6 (C-22), 27.7 (C-23), 41.9 (C-24), 85.8 (C-25), 23.3 (C-26), 22.8 (C-27), 14.4 (C-28), 21.4 (OCOCH<sub>3</sub>), 21.0(OCOCH<sub>3</sub>), 22.5(OCOCH<sub>3</sub>), 170.5(C-3-OCOCH<sub>3</sub>), 170.4(C-6-OCOCH<sub>3</sub>), 170.0(C-25-OCOCH<sub>3</sub>); EIMS [M<sup>+</sup>] 576, 516, 498, 456, 438, 312.

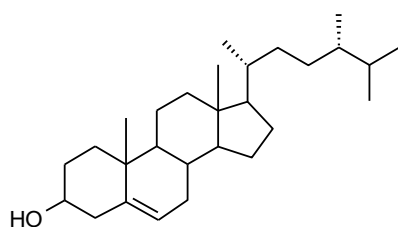
Compound **5**: Colourless needles (MeOH), m.p. 228-229°C, [α]<sub>D</sub><sup>25</sup>-48° (c, 1.0 CH<sub>3</sub>OH); analyzed for C<sub>30</sub>H<sub>52</sub>O<sub>5</sub> (calcd. C 73.1%, H 10.5%; found: 72.9% and 10.7% respectively); FTIR (CD<sub>3</sub>OD): 3423, 3412, 3373, 3258, 2938, 2931, 2870, 1739, 1442, 1369, 1253, 1045, 952cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1 and 2 in supplementary section; EIMS [M<sup>+</sup>] 492.

Compound **6**: Colourless needles (MeOH: traces of CHCl<sub>3</sub>), m.p. 242-244°C, [α]<sub>D</sub><sup>25</sup>-25° (c, 1.0 CH<sub>3</sub>OH); analyzed for C<sub>28</sub>H<sub>48</sub>O<sub>4</sub> (calcd. C 75.0%, H 10.7%; found:

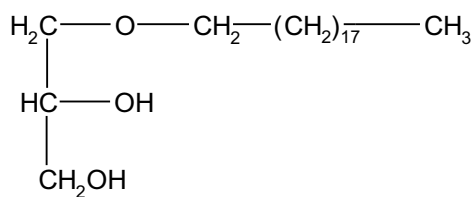
74.8% and 10.9% respectively); FTIR(CD<sub>3</sub>OD): 3490, 3433, 3317, 3304, 2935, 2838, 1442, 1375, 1284, 1045, 947cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1 and 2 in supplementary section; EIMS [M<sup>+</sup>] 448.

Compound 7: Colourless needles (MeOH), m.p. 258-259°C, [α]<sup>25</sup><sub>D</sub>-55° (c, 1.67 CH<sub>3</sub>OH); analyzed for C<sub>30</sub>H<sub>52</sub>O<sub>6</sub> (calcd. C 70.8%, H 10.2%; found: 70.6% and 10.3% respectively); FTIR (CD<sub>3</sub>OD): 3492, 3452, 2941, 2868, 1724, 1379, 1355, 1276, 1043, 958cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1 and 2 in supplementary section; EIMS [M<sup>+</sup>] 508.

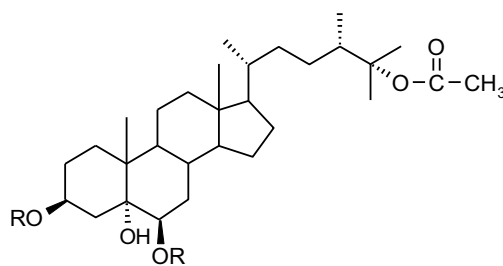
**Figure:** New and known compounds isolated from *Callyspongia fibrosa*



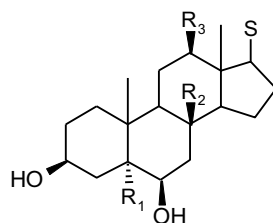
**1**



**2**



**4, a, R=H; b, R=Ac**



Compound

R<sub>1</sub>

R<sub>2</sub>

R<sub>3</sub>

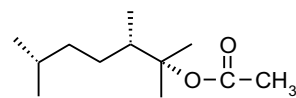
S

**3**

H

H

H

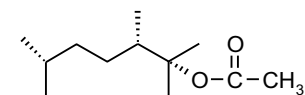


**5**

H

OH

H

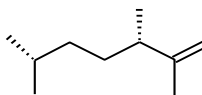


**6**

OH

H

OH

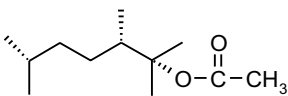


**7**

OH

H

OH



**New polyhydroxy sterols from the marine sponge *Callispongia fibrosa*  
(Ridley & Dendly)**

Thota S. Prakasa Rao, Nittala S. Sarma, Y.L.N. Murthy,  
Venkata S.S.N. Kantamreddi, Colin W. Wright, P.S. Parameswaran

**SUPPLEMENTARY SECTION**

**Table No.1.** <sup>1</sup>H NMR spectral data of sterols

Assign- ment	<b>3</b>	<b>4a</b>	<b>5</b>	<b>6</b>	<b>7</b>
3β-H	4.02 (1H, m)	3.98 (1H, m)	4.00 (1H, m)	4.00 (1H, dd)*	3.94 (1H, m) <sup>@</sup>
6β-H	3.44 (1H, br s)	3.42 (1H, br s)	3.45 (1H, br s)	3.42 (1H, br s)	3.41 (1H, br s)
12β-H				4.00 (1H, dd)*	3.94 (1H, m) <sup>@</sup>
18-H <sub>3</sub>	0.70 (3H, s)	0.71 (3H, s)	0.71 (3H, s)	0.69 (3H, s)	0.69 (3H, s)
19-H <sub>3</sub>	1.14 (3H, s)	1.14 (3H, s)	1.15 (3H, s)	1.11 (3H, s)	1.13 (3H, s)
21-H <sub>3</sub>	0.91(3H, d, 5.4Hz)	0.94 (3H, d, 5.4Hz)	0.94 (3H, d 8.1Hz)	0.99 (3H, d 6.6Hz)	0.93 (3H, d 5.4Hz)
26-H <sub>3</sub>	1.37 (3H, s)	1.38 (3H, s)	1.38 (3H, s)	4.65(2H, br s, 26-H <sub>2</sub> ) )	1.38 (3H, s)
27-H <sub>3</sub>	1.39 (3H, s)	1.40 (3H, s)	1.40 (3H, s)	1.62(3H, s)	1.40 (3H, s)
28-H <sub>3</sub>	0.89(3H, d, 5.4Hz)	0.89 (3H, d, 5.4Hz)	0.89 (3H, d 5.4Hz)	0.91 (3H, d 6.6Hz)	0.89 (3H, d 5.4Hz)
-OCOCH <sub>3</sub>	1.94 (3H, s)	1.98 (3H, s)	1.95 (3H, s)	-	1.96 (3H, s)

\*, @: Overlapping signals

**Table No.2.**  $^{13}\text{C}$  NMR spectral data of sterols

Carbon. No.	<b>3</b>	<b>4a</b>	<b>5</b>	<b>6</b>	<b>7</b>
1	30.8	30.31	30.32	30.77	30.81
2	27.0	27.93	27.84	27.82	27.88
3	67.1	67.00	64.01	64.64	64.67
4	37.9	37.99	37.99	37.99	37.01
5	40.1	75.19	40.14	75.72	75.77
6	76.1	75.48	75.48	76.15	76.19
7	34.0	34.00	36.13	34.77	34.69
8	33.8	33.96	75.20	34.01	33.87
9	43.5	41.97	45.25	41.93	41.93
10	37.9	36.18	37.99	40.62	40.64
11	21.1	21.13	21.87	21.12	21.87
12	41.8	40.13	41.13	72.98	73.01
13	41.9	45.25	41.02	43.52	46.13
14	56.1	55.13	56.13	56.45	56.20
15	24.2	24.15	24.21	24.21	23.69
16	27.5	27.93	28.14	30.91	30.81
17	55.9	45.25	56.01	56.19	56.10
18	11.3	11.32	11.32	8.84	8.88
19	16.0	16.00	16.01	11.26	11.32
20	36.1	36.16	34.06	36.15	36.24
21	17.8	17.82	17.86	17.83	18.18
22	34.1	34.11	34.08	33.84	34.69
23	27.1	27.93	27.18	27.78	27.41
24	42.0	42.59	42.59	42.08	41.97
25	86.0	86.06	86.06	149.69	86.10
26	24.0	22.49	24.01	108.89	24.25
27	27.7	27.93	27.71	27.91	27.51
28	13.5	13.56	13.59	13.52	13.51
OCO <u>C</u> H <sub>3</sub>	20.9	20.99	21.15	20.9	21.16
OCO <u>C</u> H <sub>3</sub>	--	--	--	--	--
OCO <u>C</u> H <sub>3</sub>	--	--	--	--	--
OCO <u>C</u> H <sub>3</sub>	171.1	171.10	171.11	--	171.14
OCO <u>C</u> H <sub>3</sub>	--	--	--	--	--
OCO <u>C</u> H <sub>3</sub>	--	--	--	--	--

**Table No. 3:** Antimalarial testing results of sterols

Compound	IC <sub>50</sub> (( $\mu$ M) = Mean $\pm$ SD(n) <sup>‡</sup>	
	<b>3D7*</b>	<b>K1#</b>
<b>3</b>	54.81 $\pm$ 0.95	54.02 $\pm$ 1.01
<b>4a</b>	30.10 $\pm$ 0.77	20.54 $\pm$ 0.26
<b>5</b>	48.46 $\pm$ 0.88	44.44 $\pm$ 1.09
<b>7</b>	48.48 $\pm$ 1.02	47.75 $\pm$ 0.89
Chloroquine	0.04 $\pm$ 0.02	0.72 $\pm$ 0.04

<sup>‡</sup> n=3; \*Chloroquine sensitive; # Chloroquine resistant