Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 8851–8854

# Novel ring B abeo-sterols as growth inhibitors of *Mycobacterium tuberculosis* isolated from a Caribbean Sea sponge, *Svenzea zeai*

Xiaomei Wei,[a] Abimael D. Rodríguez,[a,*] Yuehong Wang[b] and Scott G. Franzblau[b]

[a]*Department of Chemistry, University of Puerto Rico, PO Box 23346, U.P.R. Station, San Juan 00931-3346, PR, USA*
[b]*Institute for Tuberculosis Research, College of Pharmacy, The University of Illinois at Chicago, IL 60612-7231, USA*

Received 10 August 2007; revised 10 October 2007; accepted 12 October 2007
Available online 18 October 2007

**Abstract**—Assay-guided fractionation of a moderately strong anti-tubercular extract obtained from the Caribbean Sea sponge *Svenzea zeai* afforded two novel 5(6 → 7)abeo-sterols, named parguesterols A (**1**) and B (**2**), as its active components. Their structures were elucidated on the basis of extensive spectroscopic analysis.


Sponges of the class Demospongiae are known to produce a large array of interesting steroids, mainly $\Delta^5$-3β-hydroxy steroids, 3-ketosterols and rearranged sterols, steroidal alkaloids, polyhydroxysteroids, steroid peroxides, steroidal glycosides, and those with modified side chains.[1,2] However, prior to this report, there has been only one account of a steroid possessing a contracted cyclopentane B-ring from a marine organism, namely, a Japanese sponge *Stelletta hiwasaensis*.[3,4] While searching for new natural products that inhibit the growth of *Mycobacterium tuberculosis* from marine invertebrates, we isolated two novel sterols named parguesterol A (**1**) and parguesterol B (**2**). An assay-guided fractionation of an anti-tubercular extract obtained from the common Caribbean sponge *Svenzea zeai* (phylum: Porifera; class: Demospongiae; order: Halichondrida; family: Dictyonellidae) collected in La Parguera, Puerto Rico yielded **1** and **2** as its active components.[5] After extensive 2D NMR studies in combination with IR, UV, and MS analyses, their structures were revealed as novel 5(6 → 7)abeo-sterols. To our knowledge, compounds **1** and **2** constitute only the second and third examples of 6-5-6-5 fused rings sterols of marine origin to be described.[6] The MIC values for anti-tubercular activity of parguesterols A (**1**) and B (**2**) were determined as 7.8 and 11.2 μg/mL.

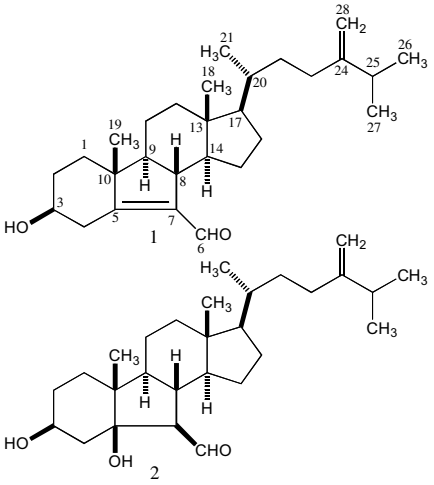
The crude methanol–chloroform extract obtained from *S. zeai* inhibited 100% of the growth of *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) at a concentration of 128 μg/mL using the Microplate Alamar Blue Assay (MABA).[7] However, at lower concentrations (64 μg/mL) the inhibitory activity of the crude extract was diminished to 84%. The hexane-soluble fraction (17.1 g) of the methanol–chloroform sponge extract (47.1 g) was chromatographed consecutively on a Bio-Beads SX-3 column with toluene as eluent and on a Si gel column using eluents of increasing polarity (hexane, EtOAc, and acetone) to yield a mixture of compounds. Fractionation was monitored using anti-tubercular activity against *M. tuberculosis* $H_{37}Rv$. The active fractions containing sterols were further purified by Si gel column chromatography with mixtures of hexane and acetone to yield compounds **1** (3.5 mg, 0.007% yield) and **2** (4.2 mg, 0.009% yield).[8]

Parguesterol A (**1**)[9] was obtained as a colorless oil whose HR-EI MS showed a molecular ion peak at *m*/*z* 412.3348 (*Δ* +0.7 mmu) corresponding to the molecular formula $C_{28}H_{44}O_2$. The IR spectrum (thin film) displayed strong bands at 3400, 1719, and 1677 $cm^{-1}$ attributed to the hydroxy, α,β-unsaturated aldehyde, and olefin, respectively. The UV spectrum showing $\lambda_{max}$ (MeOH) 237 nm (ε 17,000) confirmed the presence of a conjugated enal in **1**.[10] The $^1H$, $^{13}C$ NMR, and HSQC spectral data suggested the presence of three secondary methyls, two tertiary methyls, nine methylenes, six methines, five quaternary carbons (of which two were $sp^3$ and three were $sp^2$), one oxygenated methine, one

*Keywords*: Tuberculosis; Caribbean sponge; *Mycobacterium tuberculosis*; Abeo-sterols; *Svenzea zeai*.
* Corresponding author. Tel.: +1 787 764 0000x4799; fax: +1 787 756 8242; e-mail: abrodriguez@uprrp.edu

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.10.070

*exo*-methylene, and one aldehyde (Table 1). The $^1$H–$^1$H COSY, TOCSY, HSQC, and DEPT-135 spectra of **1** afforded three partial structures, **A** [C1–C4], **B** [C8–C17, C20–C23], and **C** [C25–C27]. The connectivities across partial structures **A**–**C** and five quaternary carbons, two tertiary methyls, α,β-unsaturated aldehyde, and *exo*-methylene were accomplished confidently with the assistance of HMBC, TOCSY, and NOESY NMR experiments as shown in Figure 1.



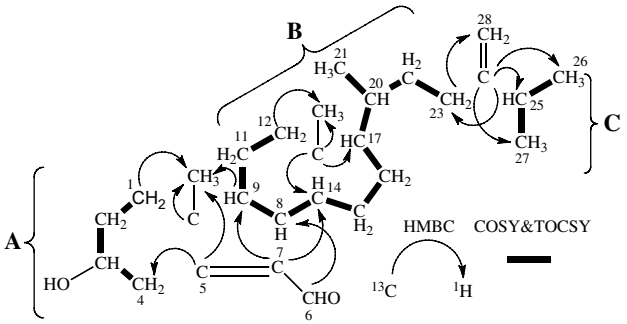



**Figure 1.** $^1$H–$^1$H COSY, TOCSY, and HMBC correlations of parguesterol A (**1**).

The relative stereochemistries at C3, C8–C10, C13, C14, C17, and C20 in **1** were assigned on the basis of the NOESY correlations (Fig. 2). Key NOE correlations for **1** showed interactions between H-3/H-4α ($\delta$ 3.47) and H-4α/H-9. Also, $H_3$-19 ($\delta$ 0.94) showed NOE responses with both H-4β and H-8. The NOE correlations of $H_3$-18 with both H-8 and H-20, but not with H-14 or H-17, confirmed the β-orientation of $H_3$-18 and thus, H-17 and $H_3$-21 should be located on the α-face. The α-configuration of the latter protons was suggested also by the strong NOEs from $H_3$-21 to H-12αβ and H-17.

**Table 1.** $^1$H (500 MHz), $^{13}$C NMR (125 MHz), and NOESY spectral data for parguesterol A (**1**) and parguesterol B (**2**)[a]

| No. | **1**; $\delta_H$ (intrgt, mult, *J* in Hz) | **1**; $\delta_C$ (mult)[b] | NOESY | **2**; $\delta_H$ (intrgt, mult, *J* in Hz) | **2**; $\delta_C$ (mult)[b] | NOESY |
|---|---|---|---|---|---|---|
| 1α | 1.90 (1H, m) | 36.2 ($CH_2$) | H-1β, H-3, H-9 | 1.69 (1H, m) | 26.8 ($CH_2$) | H1β, H-9 |
| 1β | 1.27 (1H, m) | | H-1α, $H_3$-19 | 1.37 (1H, m) | | H-1α, $H_3$-19 |
| 2αβ | 1.97 (2H, m) | 31.3 ($CH_2$) | | 1.65 (2H, m) | 28.0 ($CH_2$) | |
| 3 | 3.70 (1H, m) | 70.9 (CH) | H-1α, H-4α | 4.12 (1H, br s) | 67.4 (CH) | H-4α |
| 4α | 3.47 (1H, br d, 15.0) | 33.9 ($CH_2$) | H-3, H-4β | 2.14 (1H, m) | 44.3 ($CH_2$) | H-3, H-4β, H-7 |
| 4β | 2.08 (1H, m) | | H-4α, $H_3$-19 | 1.73 (1H, dt, 3.0, 15.0) | | H-4α |
| 5 | | 168.9 (C) | | | 84.2 (C) | |
| 6 | 9.97 (1H, s) | 189.6 (CH) | | 9.70 (1H, d, 3.0) | 204.6 (CH) | H-8 |
| 7 | | 139.3 (C) | | 2.25 (1H, m) | 63.9 (CH) | H-4α, H-9, H-14 |
| 8 | 2.55 (1H, t, 9.0) | 46.3 (CH) | $H_3$-18, $H_3$-19 | 2.12 (1H, m) | 40.0 (CH) | H-6, H-11β, $H_3$-18, $H_3$-19 |
| 9 | 1.16 (1H, m) | 60.1 (CH) | H-1α, H-12α | 1.29 (1H, m) | 50.5 (CH) | H-1α, H-7, H-14 |
| 10 | | 46.1 (C) | | | 45.5 (C) | |
| 11α | 1.48 (1H, m) | 20.7 ($CH_2$) | H-11β | 1.48 (1H, m) | 21.6 ($CH_2$) | H-11β |
| 11β | 1.39 (1H, m) | | H-8, $H_3$-18, $H_3$-19 | 1.43 (1H, m) | | H-11α, $H_3$-18, $H_3$-19 |
| 12α | 2.06 (1H, m) | 39.8 ($CH_2$) | H-9, H-17, $H_3$-21 | 2.05 (1H, m) | 39.7 ($CH_2$) | H-12β, H-14, H-17 |
| 12β | 1.12 (1H, m) | | $H_3$-18, $H_3$-21 | 1.12 (1H, m) | | H-12α, $H_3$-18, $H_3$-21 |
| 13 | | 45.3 (C) | | | 44.8 (C) | |
| 14 | 1.33 (1H, m) | 54.5 (CH) | H-9, H-17 | 1.18 (1H, m) | 56.2 (CH) | H-7, H-9, H-12α, H-15α |
| 15α | 1.74 (1H, m) | 26.6 ($CH_2$) | H-15β | 1.44 (1H, m) | 24.6 ($CH_2$) | H-14, H-15β |
| 15β | 1.54 (1H, m) | | H-8, H-15α, $H_3$-18 | 1.09 (1H, m) | | H-15α |
| 16αβ | 1.85 (2H, m) | 28.5 ($CH_2$) | | 1.86 (1H, m); 1.30 (1H, m) | 28.3 ($CH_2$) | |
| 17 | 1.14 (1H, m) | 55.2 (CH) | H-14, $H_3$-21 | 1.10 (1H, m) | 55.6 (CH) | H-12α, $H_3$-21 |
| 18 | 0.73 (3H, s) | 12.5 ($CH_3$) | H-8, H-11β, H-20 | 0.72 (3H, s) | 12.5 ($CH_3$) | H-8, H-11β, H-20 |
| 19 | 0.94 (3H, s) | 15.6 ($CH_3$) | H-1β, H-8, H-11β | 0.93 (3H, s) | 18.4 ($CH_3$) | H-1β, H-8, H-11β |
| 20 | 1.43 (1H, m) | 35.5 (CH) | $H_3$-18, $H_3$-21 | 1.41 (1H, m) | 35.6 (CH) | $H_3$-18, $H_3$-21 |
| 21 | 0.96 (3H, d, 6.2) | 18.9 ($CH_3$) | H-17, H-12αβ | 0.94 (3H, d, 6.5) | 18.7 ($CH_3$) | H-17, H-20 |
| 22αβ | 1.59 (1H, m); 1.15 (1H, m) | 34.7 ($CH_2$) | | 1.55 (1H, m); 1.12 (1H, m) | 34.7 ($CH_2$) | |
| 23αβ | 2.08 (1H, m); 1.88 (1H, m) | 31.1 ($CH_2$) | | 2.09 (1H, m); 1.88 (1H, m) | 31.0 ($CH_2$) | |
| 24 | | 156.8 (C) | | | 156.7 (C) | |
| 25 | 2.21 (1H, m) | 33.8 (CH) | $H_3$-26, $H_3$-27 | 2.21 (1H, m) | 33.8 (CH) | $H_3$-26, $H_3$-27 |
| 26 | 1.02 (3H, d, 6.5) | 22.0 ($CH_3$) | H-25 | 1.02 (3H, d, 6.5) | 22.0 ($CH_3$) | H-25 |
| 27 | 1.01 (3H, d, 6.5) | 21.9 ($CH_3$) | H-25 | 1.02 (3H, d, 7.0) | 21.8 ($CH_3$) | H-25 |
| 28αβ | 4.71 (1H, s); 4.65 (1H, s) | 106.0 ($CH_2$) | | 4.71 (1H, s); 4.65 (1H, s) | 106.0 ($CH_2$) | |

[a] Spectra were recorded in $CDCl_3$ at 25°C. Chemical shift values are in parts per million relative to TMS.
[b] $^{13}$C NMR multiplicities were obtained from a DEPT-135 experiment.

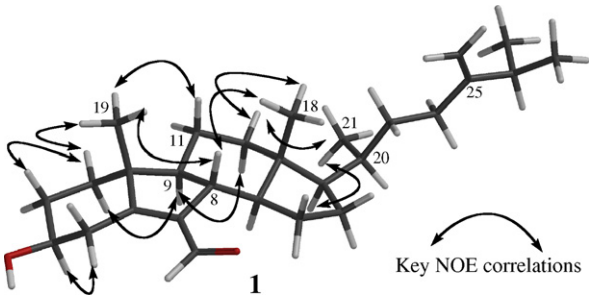



**Figure 2.** Relative stereochemistry of parguesterol A (**1**).

Parguesterol B (**2**)[11] was isolated as a UV inactive colorless oil. Its molecular formula, $C_{28}H_{46}O_3$, was established by HR-EI MS (*m/z* 430.3443, $[M]^+$), implying six degrees of unsaturation. The presence of two hydroxy groups was suggested by a strong absorption band at 3434 $cm^{-1}$ in the IR spectrum (thin film) and further supported by the ion peaks at *m/z* 412 $(M–H_2O)^+$ and 394 $(M–2H_2O)^+$ in the EI MS spectrum. In addition, the IR spectrum displayed strong bands at 2736 and 1715 $cm^{-1}$ ascribable to an aldehyde carbonyl group. The $^{13}C$ NMR spectral data of **2** (Table 1) indicated the presence of 28 carbon atoms, including five methyls, nine $sp^3$ methylenes, one $sp^2$ methylene, eight $sp^3$ methines (including one oxygenated), four quaternary carbons (including one $sp^2$ and one oxygenated), and one aldehyde. In general, the spectroscopic data of **2** (IR, $^1H$, and $^{13}C$ NMR) were similar to those of **1**, except for the absence of the 5,7-olefin signals, which were replaced by signals of a carbon–carbon single bond [$\delta$ 84.2 (C, C5) and 63.9 (CH, C7)] in **2**. This was further confirmed by HMBC correlations observed from $H_3$-19 ($\delta$ 0.93, s) to C5 ($\delta$ 84.2) and from H-6 ($\delta$ 9.70, d, $J = 3.0$ Hz) to both C7 ($\delta$ 63.9) and C5. Moreover, careful comparison of the NMR and UV data of **1** with those of **2** revealed that the α,β-unsaturated aldehyde constellation across C5–C7 on **1** was replaced by a β-hydroxy aldehyde moiety in **2**. The relative stereochemistry of parguesterol B (**2**) was established by NOESY correlations in comparison with those of **1**. The NOE correlations observed between H-7 ($\delta$ 2.25) and methines H-4α ($\delta$ 2.14), H-9 ($\delta$ 1.29), and H-14 ($\delta$ 1.18) confirmed the α-orientation of H-7. Additional key NOE correlations for **2** showed interactions between H-6/H-8 and H-8/$H_3$-19. Hence, H-6 and the C5 hydroxy group must be positioned on the β-face.

Tuberculosis (TB) continues to be the single largest infectious killer disease in the world.[12] Certain sterols of structure similar to compounds **1** and **2** have been reported to show potent anti-tuberculosis activity suggesting that this class of triterpenoids hold great promise as anti-mycobacterial agents.[13,14] Therefore, compounds **1** and **2** were assayed for anti-tuberculosis activity against *M. tuberculosis* $H_{37}Rv$. Studies aimed at understanding their mode(s) of action have shown that the minimum structural requirement for sterols to be involved in mycobacterial cell wall disruption include a polar head group and a flexible non-polar phytyl tail.[15] Thus, parguesterol A (**1**) and parguesterol B (**2**) constitute important lead structures for the development of novel tuberculosis drugs due to their strong activity, specificity, and low toxicity against Vero cells ($IC_{50}$ values = 52 μg/mL).[16] More importantly, the 5(6 → 7)abeosteroidal nucleus present in compounds **1** and **2** could represent a novel scaffold for the development of new anti-tuberculosis agents.[17] In order to test this hypothesis, further work on the synthesis and biological screening of additional 6-5-6-5 fused rings sterol analogs from representative sterols possessing the usual 3β-hydroxy-$\Delta^5$-cholestane nucleus is in progress.[18]

## Acknowledgments

Field and bench work for this project was supported in part, by funding from the NIH-SCORE Program (Grant S06GM08102) of the University of Puerto Rico, Río Piedras Campus. The authors would also like to express their sincere gratitude to Mr. Jan Vicente for the collection and taxonomic identification of the sponge specimen.

## Supplementary data

The structures and isolation yields of known sterols **3**–**8** co-isolated from *S. zeai* during this investigation, and relevant literature references. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.070.

## References and notes

1. Sarma, N. S.; Krishna, M. S. R.; Rao, S. R. *Mar. Drugs* **2005**, *3*, 84–111.
2. Kerr, R. G.; Baker, B. J. *Nat. Prod. Rep.* **1991**, *8*, 465–498.
3. Miyamoto, T.; Kodama, K.; Aramaki, Y.; Higuchi, R.; Van Soest, R. W. M. *Tetrahedron Lett.* **2001**, *42*, 6349–6351.
4. Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31–86, and previous articles in this series.
5. The sponge *Svenzea zeai* was formerly known as *Calyx podatypa*, see: (a) Alvarez, B.; Van Soest, R. W. M.; Rützler, K. *Contrib. Zool.* **2002**, *71*, 171–176; For prior chemical work with this sponge, see: (b) Doss, G. A.; Djerassi, C. *J. Am. Chem. Soc.* **1988**, *110*, 8124–8128; (c) Rodríguez, A. D.; Cóbar, O. M.; Padilla, O. L. *J. Nat. Prod.* **1997**, *60*, 915–917; (d) Rodríguez, A. D.; Cóbar, O. M.; Padilla, O. L. *J. Nat. Prod.* **1997**, *60*, 1331–1333; (e) Carballeira, N. M.; Pagán, M.; Rodríguez, A. D. *J. Nat. Prod.* **1998**, *61*, 1049–1052.
6. To date, the only other reported source of 6-5-6-5 fused rings sterols is the Taiwanese plant *Taiwania cryptomerioides*, see: Lin, W.-H.; Fang, J.-M.; Cheng, Y.-S. *Phytochemistry* **1998**, *48*, 1392–1397.
7. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
8. Besides the new abeo-sterols **1** and **2**, our specimen contained a complex mixture of sterols which, after careful purification and spectroscopic analysis, were identified as the known sterols **3**–**8** (see Supplementary data).
9. Parguesterol A (**1**): colorless oil; $[\alpha]_D^{25}$ −4.4 (*c* 1.0, $CHCl_3$); $\nu_{max}$ (thin film) 3400, 3080, 2956, 2869, 2730, 1719, 1677, 1462, 1380, 1261, 1071, 1054, 915, 886,

734 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 237 nm ($\varepsilon$ 17,000); $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz) (see Table 1); EIMS $m/z$ $[M]^+$ 412 (18), 394 (6), 328 (13), 285 (20), 257 (15), 145 (20), 95 (50), 85 (54), 83 (56), 81 (53), 69 (92), 57 (100); HREIMS $m/z$ $[M]^+$ calcd for $C_{28}H_{44}O_2$ 412.3341, found 412.3348.

10. Lambert, J. B.; Shurvell, H. F.; Lightner, D. A.; Cooks, R. G. *Organic Structural Spectroscopy*; Prentice Hall: Upper Saddle River, NJ, 1998, pp 304–344.
11. Parguesterol B (**2**): colorless oil; $[\alpha]_D^{25}$ +9.2 (*c* 1.0, $CHCl_3$); $\nu_{max}$ (thin film) 3434, 3083, 2950, 2869, 2736, 1715, 1462, 1382, 1261, 1169, 1079, 1019, 970, 910, 803, 734 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz) (see Table 1); EIMS $m/z$ $[M]^+$ 430 (4), 412 (8), 394 (3), 128 (43), 285 (12), 173 (17), 135 (26), 128 (43), 110 (62), 95 (47), 81 (61), 57 (58), 55 (100); HREIMS $m/z$ $[M]^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3443.
12. Drahl, C. *Chem. Eng. News* **2007**, *September 24*, 39–43.
13. Cantrell, C. L.; Franzblau, S. G.; Fisher, N. H. *Planta Med.* **2001**, *67*, 685–694.
14. Rugutt, J. K.; Rugutt, K. J. *Nat. Prod. Lett.* **2002**, *16*, 107–113.
15. Davidson, S. M. K.; Liu, Y.; Regen, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 10104–10110.
16. The $IC_{50}$ value of the control drug used (rifampin) in the cytotoxicity assay was 89.3 μg/mL.
17. Copp, B. R.; Pearce, A. N. *Nat. Prod. Rep.* **2007**, *24*, 278–297.
18. Interestingly, known sterol **3**, obtained as the main isolate from the same hexane-soluble fractions as compounds **1** and **2**, displayed only marginal inhibitory activity (46%) against *M. tuberculosis* $H_{37}Rv$ at a concentration of 128 μg/mL. Since **3** is the most plausible biosynthetic precursor to parguesterols A and B, this finding suggests that in active steroids, maximum anti-mycobacterial activity could be attained upon contracting the cyclohexane B-ring, most likely as a result of increasing both the hydrophilic impact and rigidity of the steroidal backbone.