Rhabdaprovidines D–G, Four New 6,6,5-Tricyclic Terpenoids from the Vietnamese Sponge Rhabdastrella providentiae

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Four new 6,6,5-tricyclic terpenoids, named rhabdaprovidines D–G (1–4), were isolated from the Vietnamese sponge Rhabdastrella providentiae. The structures of 1–4 were elucidated by extensive analysis of HR-ESI-MS and NMR spectroscopy. The absolute stereochemistry of compounds 3 and 4 were determined by both experimental and theoretical calculated circular dichroism (CD) spectra. Compounds 1, 2, and 4 contained trans-syn-trans 6,6,5-tricyclic nucleus meanwhile compound 3 possessed trans-syn-cis ring junction. Compound 4 displayed novel structure by the presence of five rings and nine chiral carbon centers in the isomalabaricane triterpene backbone.

Keywords: Rhabdastrella providentiae, Rhabdaprovidine D, Rhabdaprovidine E, Rhabdaprovidine F, Rhabdaprovidine G, 6,6,5-Tricyclic terpenoid.

Rhabdastrella sponges are a rich source of isomalabaricane-type triterpenes, which are characterized by a trans-syn-trans 6,6,5-tricyclic nucleus that is rarely found in nature [1]. The unique chemical structure of isomalabaricane analogs, and their narrow distribution in sponges make them excellent chemotaxonomic markers for Rhabdastrella sponges and a few additional genera [1b, 2]. Many isomalabaricane analogs are cytotoxic to various human cancer cell lines [2c, 3]. A previous report, three new acylated tricyclic terpenoids (rhabdaprovidines A-C) were isolated from the Vietnamese sponge R. providentiae [4]. Rhabdaprovidines A-C shared the same 6,6,5-tricyclic nucleus, but their side chain could be formed by bio-chemical degeneration of original isomalabaricane type triterpene [4a]. In our continuing efforts to find new 6,6,5-tricyclic terpenes, we report herein the isolation and structural elucidation of four tricyclic terpenoids, named rhabdaprovidines D–G (1–4), from the sponge R. providentiae (Figure 1).

Compound 1, colorless oil, had molecular formula C21H30O4 as deduced by a quasi-molecular ion peak at m/z 347.2214 [M+H]+ (calcd for C21H31O4, 347.2222) in the HR-ESI-MS, indicating 7 indices of hydrogen deficiency. The 1H-NMR spectrum of 1 contained signals for one methoxy group at δH 3.81 (3H, s); five methyl groups at δH 0.86, 1.06, 1.12, 1.38, and 2.02 (each 3H, s); and several aliphatic proton signals at δH 1.52–2.70. Among them, the geminal and vicinal coupled protons were recognized in the HSQC and COSY spectra, such as H-1 (δH 1.52 and 2.13)/H-2 (δH 2.39 and 2.70), H-5 (δH 2.40)/H-6 (δH 1.56 and 1.63)/H-7 (δH 2.03 and 2.24), H-9 (δH 1.93)/H-11 (δH 2.17 and 2.22) (Figure 2). The 13C-NMR spectrum of 1 contained signals corresponding to 21 carbon atoms that were identified by DEPT spectra as eight non-protonated carbons, five methylenes, two methines, and six methyl carbon atoms. Among the five sp3 hybridized carbons, three signals at δC 218.7, 203.2, 171.7 were assigned to carbonyl carbons and two signals at δC 146.0, 134.6 suggested the presence of one C-C double bond. Hence, seven indices of hydrogen deficiency in the molecular formula of 1 indicated the presence of four double bonds and three rings in its chemical structure. Next, HMBC correlations between H-17 (δH 0.86) and C-1 (δC 31.3)/C-5 (δC 45.4)/C-9 (δC 48.4)/C-10 (δC 34.9)/H-2 (δH 2.39, 2.70) and ketone carbonyl carbon C-3 (δC 218.7), H-18 (δH 1.06)/H-19 (δH 1.12) and C-3 (δC 46.9)/C-5 (δC 45.4), H-20 (δH 1.38) and C-7 (δC 35.3)/C-8 (δC 43.0)/C-9 (δC 48.4) indicated that two six-membered rings, A and B, share a C-5/C-10 single bond. Similarly, HMBC correlations between H-11 (δH 2.22) and ketone carbonyl carbon C-12 (δC 203.2), H-20 (δH 1.38) and quaternary olefinic carbon C-13 (δC 146.0) supported the presence of a five-membered ring C. The presence of a double bond at C-13/C-14 and a methacryboxy group at C-14 were also confirmed by HMBC correlations between H-16 (δH 2.02) and C-13 (δC 146.0)/C-14 (δC 134.6)/C-15 (δC 171.7), methoxy proton (δH 3.81) and C-15. The stereochemistry of 1 was identified by close examination of its NOESY spectrum (Figure 2). Specifically, NOESY correlations H-17 (δH 0.86)/H-18 (δH 1.06) and H-5 (δH 2.40)/H-19 (δH 1.12) suggested a trans-fused connection between rings A and B. Also, NOESY correlation between H-17 (δH 0.86) and H-9 (δH 1.93) was supported the syn-orientation of C-17 and H-9. Furthermore, the NOESY correlation H-5 (δH 2.40)/H-20 (δH 1.38) and the absence of a NOESY correlation H-20 (δH 1.38)/H-9 (δH 1.93) indicated a trans-fused connection between rings B and C.
A Z-configuration at the double bond C-13/C-14 was deduced by the NOESY correlation between H-16 (δH 2.02) and H-20 (δH 1.38). Consequently, chemical structure of compound 1 was established and named as rhabdaprovidine D.

Analysis of HR-ESI-MS of compound 2 showed a quasi-molecular ion peak at m/z 265.1798 [M+H]+ (calcd for C14H25O3, 265.1804) in the HR-ESI-MS, exhibited signals for four methyl groups at δH 1.06, 1.15, 1.29, 1.62 (each 3H, s), and other aliphatic protons signals at δH 1.49–2.68, in the 1H-NMR spectrum. Neighboring protons were identified from HSQC and COSY experiments. The 1H-NMR spectrum of 2 contained signals corresponding to 19 carbons, with the loss of a methoxy carbon and a carbonyl carbon compared to 1. These data suggest that the methoxycarbonyl group in 1 was replaced by a hydroxyl group in 2 (Figure 1). The position of a hydroxyl group at C-14 was further confirmed by HMBC correlations between H-16 (δH 2.02) and H-20 (δH 1.38), and a deshielded chemical shift of C-14 (δC 172.4), which may indicate intramolecular hydrogen bonding between this hydroxyl group and a ketone group at C-14. Moreover, the presence of a hydroxyl group at C-14 is consistent with the deshielded signal in the 1H-NMR spectrum (δH 13.65), which may indicate intramolecular hydrogen bonding between this hydroxyl group and a ketone group at C-14. As with compound 1, NOESY correlations between H-17 (δH 0.85) and H-9 (δH 1.88) in 1 and H-18 (δH 1.05) in 2, and H-9 (δH 1.11) in H-16 (δH 1.99) confirmed a trans-syn-trans junction in the tricyclic system and a Z-configuration of the double bond at C-13/C-14 (Figure 2). Thus, compound 2 was determined to be a new 6,6,5-tricyclic terpenoid and named as rhabdaprovidine E.

Compound 3, C16H24O3 quasi-molecular ion peaks at m/z 265.1798 [M+H]+ (calcd for C16H25O3, 265.1804) in the HR-ESI-MS, exhibited signals for four methyl groups at δH 1.06, 1.15, 1.29, 1.62 (each 3H, s), and other aliphatic protons signals at δH 1.49–2.68, in the 1H-NMR spectrum. Neighboring protons were identified from HSQC and COSY analysis as H-1 (δH 1.60 and 1.64), H-2 (δH 2.39 and 2.68), H-5 (δH 1.59), H-6 (δH 1.49 and 1.63), H-7 (δH 1.58 and 2.06), and H-9 (δH 2.43) in H-11 (δH 2.43) and 2.57. The 13C-NMR and DEPT spectra of 3 contained signals corresponding to 16 carbons, including two carbonyl groups at δC 154.9 and 174.6, one oxygenated tertiary carbon at δC 85.3, and 13 aliphatic carbon signals at δC 20.2–55.6. These data and its indices of hydrogen deficiency suggested that compound 3 also contains three rings in its chemical structure. Similar to compounds 1 and 2, the HMBC correlations between H-17 (δH 1.29) and C-1 (δC 36.2), H-4 (δC 46.5), H-9 (δC 55.6), and H-2 (δC 35.4), H-2 (δC 2.68) and ketone
The molecular formula of compound 4 was determined to be C_{30}H_{46}O_{4} by a quasi-molecular ion peak at m/z 493.3289 [M+Na]⁺ (calcld for C_{30}H_{48}O_{4}Na, 493.3294) in the HR-ESI-MS. The 1H-NMR spectrum of 4 contained signals corresponding to seven methyl groups at δ_{H} 0.95, 1.05, 1.09, 1.49, 1.61, 1.68, 1.71, two olefinic protons at δ_{H} 5.10, 5.81, and three oxygenated methines at δ_{C} 41.5, 42.4, 4.70. The 13C-NMR of 4 contained signals corresponding to 30 carbon atoms that were preliminary assigned, according to the same of HSQC, HMBC, COSY, and NOESY experiments.

Table 2. 1H (500 MHz) and 13C NMR (125 MHz) data for compound 4 in CDCl₃

<table>
<thead>
<tr>
<th>No.</th>
<th>δ_{C}</th>
<th>δ_{H} (mult., J in Hz)</th>
<th>No.</th>
<th>δ_{C}</th>
<th>δ_{H} (mult., J in Hz)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>32.9</td>
<td>1.73 (m)/ 2.57 (m)</td>
<td>16</td>
<td>68.1</td>
<td>4.70 (br dd, 6.5, 8.5)</td>
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<tr>
<td>33.3</td>
<td>2.32 (m)/ 2.70 (m)</td>
<td>17</td>
<td>121.8</td>
<td>5.81 (d, 8.5)</td>
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</tr>
<tr>
<td>2</td>
<td>219.9</td>
<td></td>
<td>18</td>
<td>49.8</td>
<td>2.65 (d, 4.5)/ 2.76 (d, 4.5)</td>
</tr>
<tr>
<td>4</td>
<td>47.2</td>
<td>-</td>
<td>19</td>
<td>24.1</td>
<td>0.95 (s)</td>
</tr>
<tr>
<td>5</td>
<td>45.7</td>
<td>2.48 (dd, 2.5, 1.25)</td>
<td>20</td>
<td>141.9</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>19.4</td>
<td>1.41 (m)/ 1.58 (m)</td>
<td>21</td>
<td>16.6</td>
<td>1.71 (s)</td>
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<tr>
<td>34.4</td>
<td>1.40 (m)/ 2.04 (m)</td>
<td>22</td>
<td>39.8</td>
<td>2.06 (m)/ 2.11 (m)</td>
<td></td>
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<tr>
<td>8</td>
<td>42.6</td>
<td>-</td>
<td>23</td>
<td>26.6</td>
<td>2.08 (m)/ 2.14 (m)</td>
</tr>
<tr>
<td>9</td>
<td>54.1</td>
<td>1.90 (d, 3.0)</td>
<td>24</td>
<td>124.1</td>
<td>5.10 (t, 6.5)</td>
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<tr>
<td>10</td>
<td>36.0</td>
<td>-</td>
<td>25</td>
<td>131.6</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>77.0</td>
<td>4.24 (br s)</td>
<td>26</td>
<td>25.7</td>
<td>1.68 (s)</td>
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<tr>
<td>12</td>
<td>76.3</td>
<td>2.32 (m)/ 2.70 (m)</td>
<td>27</td>
<td>17.8</td>
<td>1.61 (s)</td>
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<tr>
<td>13</td>
<td>54.9</td>
<td>1.72 (d, 5.5)</td>
<td>28</td>
<td>29.3</td>
<td>1.09 (s)</td>
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<tr>
<td>14</td>
<td>54.0</td>
<td>-</td>
<td>29</td>
<td>19.4</td>
<td>1.05 (s)</td>
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<tr>
<td>15</td>
<td>36.4</td>
<td>1.13 (br, d 13.5)</td>
<td>30</td>
<td>36.5</td>
<td>1.49 (s)</td>
</tr>
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</table>

Assignment were done by HSQC, HMBC, COSY and NOESY experiments.

Experimental

General: Optical rotation, Jasso P2000 polarimeter; CD, Characin spectrometer; NMR, Bruker AM500 FT-NMR spectrometer; HR-ESI-MS, Agilent 6530 Accurate Mass Q-TOF LC/MS system.

Sponge material: Sponge samples of *Rhodastrella providentiae* (Dendy, 1916), collected by scuba at the sea area of Con Co, Quango Tri, Vietnam in January 2016, were identified (Voucher specimen No. HM06.2016-01, deposited at the Institute of Marine Biochemistry, VAST) by Prof. Do Cong Thong.

Extraction and isolation: The fresh sponge material (30 kg) was cut into small pieces and extracted with MeOH for three times (each 50 L, 2 hrs. in ultrasonic bath, room temperature). The Methanolic extract (420 g) was suspended in water and partitioned with dichloromethane. The dichloromethane soluble extract (200 g) was then subjected on a silica gel column chromatography (CC), eluting with dichloromethane/methanol (0-100% volume of methanol) to give four fractions D1-D4. Fraction D4 (45.0 g) was repeatedly chromatographed on reversed phase silica gel (RP-18) column and eluted with methanol/water (4/1, v/v) to give five fractions D4A-D4E. Fraction D4B was chromatographed on a silica gel CC eluting with dichloromethane/ethyl acetate (5/1, v/v) to give four smaller fractions D4B1-D4B4. Fraction D4B2 was repeatedly loaded on another silica gel column chromatography (CC), eluting with dichloromethane/methanol (10/1, v/v) to obtain compound 4 (14 mg). Fraction D4B3 was also subjected on a silica gel CC, eluting with dichloromethane/ethyl acetate (10/1, v/v) to give five fractions D4C1-D4C6. Fractions D4C2 and D4C4 were purified on a RP-18 column, eluting with acetonitrile/water (4/1,
v/v) to give compound 3 (8 mg) and compound 1 (7 mg), respectively.

Rhabdaprovidine D (1)
Colorless oil.
[α]D
25
= -18.5 (c 0.1, CHCl3).
1H-NMR and 13C-NMR are given in the Table 1.


Rhabdaprovidine E (2)
Colorless oil.
[α]D
25
= -22.7 (c 0.1, CHCl3).
1H-NMR and 13C-NMR are given in the Table 1.


Rhabdaprovidine F (3)
Colorless oil.
[α]D
25
= +17.1 (c 0.1, CHCl3).

CD (MeOH) mδCD20: -13.2(213) to -6.2(2300).
1H-NMR and 13C-NMR are given in the Table 1.


Rhabdaprovidine G (4)
Yellow oil.
[α]D
25
= +47.2 (c 0.1, CHCl3).

CD (MeOH) mδCD20: +11.3(2100), +6.1(2920).
1H-NMR and 13C-NMR are given in the Table 2.


Theoretical calculation of CD spectra for compounds 3 and 4:
Conformational searches were carried out on Spartan 14 program (Wavefunction Inc., Irvine, CA, USA). Possible conformations were optimized and subjected to TDDFT calculation on Gaussian 09 program [7]. The calculated ECD spectra were composed after correction based on the Boltzmann distribution of the stable conformers using SpecDis v.1.64 software [8]. Each stereoisomer

3a: 55,8S,9R,10R, (b): 5R,8R,9S,10S, (4a): 5R,8S,9R,10R,11R,12R,13R,14R,16R) were submitted to conformational searches at the Molecular Mechanics MMFF set. The initial stable conformers for each enantiomer (6 conformers for 3a, 3b, and 18 conformers for 4a, 4b) with Boltzmann distributions over 1% were further optimized by DFT calculations at the B3LYP/6-31G(d,p) basis set. The solvent effects were taken by a polarizable continuum model (PCM) calculation with methanol as the solvent. The optimized conformers were then subjected to TDDFT calculations at the B3LYP/6-31G(d,p) level in the presence of methanol with a PCM. The ECD spectra at 30 exciton states for each conformers were collected and summed to obtain theoretical ECD spectra of each enantiomer. The half-bands were taken at ζ = 0.3 eV for each enantiomers.

Supplementary data: HR-ESI-MS, NMR, and CD spectra of compounds 1-4 can be found in the online version.

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References