The Journal of Antibiotics (2014) 67, 187–189

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# New doramectin analogs from mutant *Streptomyces avermitilis* NEAU1069-3

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*The Journal of Antibiotics* (2014) **67,** 187–189; doi:10.1038/ja.2013.97; published online 9 October 2013

**Keywords:** doramectin analogs; mutant strain; *Streptomyces avermitilis* NEAU1069

Microbial metabolites used as potential pesticides have attracted great interest from the agricultural and food community due to their potential activity and low toxicity.[1,2] Several microbial metabolites, such as the avermectin and milbemycin families, have been proven to be potent preventatives and treatment against a variety of pests such as insects and parasites. During the course of our screening program for new natural pesticides and antiparasitic veterinary drugs, two novel macrocyclic lactones, three milbemycins and six new doramectin congeners have been isolated from *Streptomyces avermitilis* NEAU1069.[3–6] In the effort to improve the doramectin yield, a mutant *S. avermitilis* NEAU1069-3 was obtained through the treatment of the spores of *S. avermitilis* NEAU1069 with UV and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Compared with the wild-type strain, *S. avermitilis* NEAU1069-3 showed significantly different phenotypes such as the morphology of aerial mycelia and the metabolite HPLC profiles. Therefore, the secondary metabolites of mutant *S. avermitilis* NEAU1069-3 were investigated, leading to two new doramectin analogs **1** and **2** (Figures 1 and 2). The discovery of new doramectin analogs **1** and **2** in the mutant *S. avermitilis* NEAU1069-3 may shed new insight into the biosynthesis of doramectins. Here we described the fermentation, isolation and structural elucidation of these two new doramectin analogs.

The culture and fermentation of mutant *S. avermitilis* NEAU1069-3 were conducted according to the procedure as described in the literature.[5] The fermentation broth (30 liters) was filtered. The resulting cake was washed with water, and both filtrate and wash were discarded. Methanol (10 liters) was used to extract the washed cake. The MeOH extract was evaporated under reduced pressure to 2 liters at 45 °C and the resulting concentrate was extracted three times using an equal volume of EtOAc. The combined EtOAc phase was concentrated under reduced pressure to yield 26 g of oily substances. The residual oily substance was chromatographed on silica gel (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) and eluted with a petroleum ether–acetone mixture (100:0–50:50, v/v). The fractions eluted with the petroleum ether–acetone mixture (90:10, v/v) were combined and evaporated to obtain fraction I, and the fractions eluted with the petroleum ether–acetone mixture (85:15, v/v) were pooled and concentrated to give fraction II. The fraction I was subjected to Sephadex LH-20 (GE Healthcare, Glies, UK) gel column eluting with MeOH to give subfraction I. The semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm i.d.; Agilent, Palo Alto, CA, USA) was applied to obtain pure compounds. The eluates were monitored using a photodiode array detector at 254 nm, and the flow rates were 1.5 ml min$^{-1}$ at room temperature. The subfraction I was further separated by semi-preparative HPLC using a solvent containing a $CH_3OH$–$H_2O$ mixture (90:10, v/v) to obtain compound **1** ($t_R$ 14.5 min, 17 mg). The fraction II was subjected to Sephadex LH-20 gel column eluting with MeOH to give subfraction II, which was subsequently purified by semi-preparative HPLC using a solvent containing a $CH_3OH$–$CH_3CN$–$H_2O$ mixture (48:45:7, v/v/v) to obtain compound **2** ($t_R$ 24.5 min, 13 mg).

Compound **1** (Figure 1) was obtained as colorless oil. Its molecular formula was determined to be $C_{43}H_{60}O_9$ on the basis of HRESIMS (found: *m/z* 743.4093 $[M+Na]^+$, calculated for $C_{43}H_{60}O_9Na$, 743.4130), indicating 14 degrees of unsaturation. The IR spectrum of **1** showed absorption bands assignable to the hydroxyl group (3414 cm$^{-1}$) and carbonyl group (1703 cm$^{-1}$). The $^1$H NMR (400 MHz, $CDCl_3$) data (Table 1) showed two downfield proton signals at $\delta_H$ 7.39 (1H, s), 6.62 (1H, s), one trans-double bond at $\delta_H$ 6.09 (1H, dd, $J$ = 15.0, 10.0 Hz), 5.46 (1H, dd, $J$ = 15.0, 10.0 Hz), one methoxy group at $\delta_H$ 3.53 (3H, s), an aromatic methyl at $\delta_H$ 2.24 (3H, s), two olefinic methyls at $\delta_H$ 2.07 (3H, br s), 1.57 (3H, br s), and three aliphatic doublet methyls at $\delta_H$ 1.28 (3H, d, $J$ = 6.2 Hz), 1.17 (3H, d, $J$ = 6.9 Hz) and 0.92 (3H, d, $J$ = 7.2 Hz). Its $^{13}$C NMR and DEPT data (Table 1) displayed an ester carbonyl at $\delta_C$ 169.7 (s), a ketal at $\delta_C$ 95.9 (s), an acetal at $\delta_C$ 95.0 (d), one methoxy carbon at $\delta_C$ 56.8 (q), seven oxygenated methines at $\delta_C$ 83.0 (d), 78.3 (d), 77.3

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Received 9 June 2013; revised 28 August 2013; accepted 9 September 2013; published online 9 October 2013

(d), 76.2 (d), 68.5 (d), 67.9 (d) and 67.9 (d), three aliphatic methines at $\delta_C$ 38.7 (d), 30.1 (d) and 41.0 (d), six methyls at $\delta_C$ 15.3 (q), 18.2 (q), 19.6 (q), 17.7 (q), 15.6 (q), and 16.6 (q) in addition to nine aliphatic methylenes and 14 $sp^2$ carbons. Comparison of the $^1$H and $^{13}$C NMR data of **1** with those of the doramectin analog (**3**, Figure 1) reported in the literature[5] suggested that **1** was similar to **3**. The difference between **1** and **3** is that a double bond was present in C-22 and C-23 in **1**, which is supported by the 18 mass unit difference from **3**. The $^1$H–$^1$H COSY correlation (Figure 1) of $\delta_H$ 5.54 and $\delta_H$ 5.73, and the observed HMBC correlation from C-24 methyl group ($\delta_H$ 0.92) to $\delta_C$ 136.1 (C-23; Figure 1) further confirmed the structural assignment of **1**. As a result, the gross structure of **1** was established as shown in Figure 1. The relative stereochemistry was assigned by analogy with **3**.

Compound **2** (Figure 2) was also obtained as colorless oil with $[\alpha]_D^{25}$ +72.7° (*c* 0.08, EtOH). Its molecular formula was determined to be $C_{44}H_{66}O_{12}$ by HRESIMS (found: *m/z* 809.4445 $[M+Na]^+$, calculated for $C_{44}H_{66}O_{12}Na$, 809.4446). The IR spectrum showed absorption bands due to the hydroxyl group (3506 cm$^{-1}$) and carbonyl group (1734 cm$^{-1}$). The $^1$H and $^{13}$C NMR spectra in connection with HMQC experiment of **2** showed 64 proton and 44 carbon signals, and the multiplicity of carbon signals was classified into one carbonyl ($\delta$ 173.8), three $sp^2$ quaternary carbons, five $sp^2$ methines, one ketal ($\delta$ 99.7), one acetal ($\delta$ 95.0), an oxygen-bearing quaternary carbon ($\delta$ 80.6), an oxygen-bearing methylene ($\delta$ 68.2), ten oxygenated methines, two methoxy carbons ($\delta$ 57.8, 56.6), four $sp^3$ methines and five methyl carbons in addition to ten aliphatic methylenes by analysis of HMQC data. All the carbons and the corresponding proton signals were assigned by extensive analysis of the HMQC spectrum. The similarity of the NMR data between **2** and the known compound (**4**, Figure 2)[5] and selamectin[7] indicated that **2**

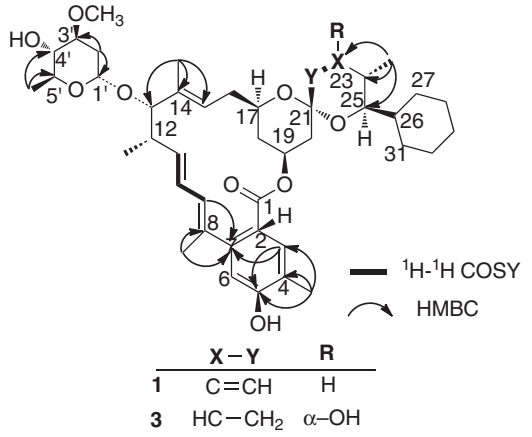


**Figure 1** Structures of **1**, **3** and key $^1$H–$^1$H COSY and HMBC correlations of **1**.

**Table 1 $^{13}$C (100 MHz) and $^1$H (400 MHz) NMR assignments for 1 and 2 in $CDCl_3$**

| | $\delta_H$ (*J* in Hz) | | $\delta_C$ (p.p.m.) | |
|---|---|---|---|---|
| *Position* | **1** | **2** | **1** | **2** |
| 1 | | | 169.7 (s) | 173.8 (s) |
| 2 | | 3.33 (1H, br s) | 123.7 (s) | 45.6 (d) |
| 3 | 7.39 (1H, s) | 5.40 (1H, br s) | 132.1 (d) | 118.3 (d) |
| 4 | | | 122.6 (s) | 136.2 (s) |
| 5 | | 3.96 (1H, br s) | 155.8 (s) | 76.9 (d) |
| 6 | 6.62 (1H, s) | 4.03 (1H, br s) | 114.1 (d) | 77.4 (d) |
| 7 | | | 144.2 (s) | 80.6 (s) |
| 8 | | | 134.6 (s) | 139.8 (s) |
| 8a | | 4.63 (1H, dd, 14.5, 2.2)<br>4.70 (1H, dd, 14.5, 2.2) | | 68.2 (t) |
| 9 | 5.73 (1H, d, 10.0) | 5.84 (1H, m) | 128.5 (d) | 119.7 (d) |
| 10 | 6.09 (1H, dd, 15.0, 10.0) | 5.74 (1H, overlapped) | 126.9 (d) | 124.8 (d) |
| 11 | 5.46 (1H, dd, 15.0, 10.0) | 5.74 (1H, overlapped) | 135.8 (d) | 137.7 (d) |
| 12 | 2.53 (1H, m) | 2.53 (1H, m) | 41.0 (d) | 39.7 (d) |
| 13 | 4.00 (1H, br s) | 3.96 (1H, br s) | 83.0 (d) | 81.6 (d) |
| 14 | | | 134.1 (s) | 135.6 (s) |
| 15 | 4.94 (1H, br d, 10.6) | 4.97 (1H, br t, 7.4) | 118.7 (d) | 117.5 (d) |
| 16 | 2.28 (1H, m)<br>2.39 (1H, m) | 2.31 (2H, m) | 33.6 (t) | 34.2 (t) |
| 17 | 3.99 (1H, m) | 3.74 (1H, m) | 68.5 (d) | 68.2 (d) |
| 18 | 0.76 (1H, q, 11.9)<br>1.95 (1H, m) | 0.88 (1H, m)<br>1.81 (1H, m) | 36.8 (t) | 36.5 (t) |
| 19 | 5.56 (1H, m) | 5.35 (1H, m) | 67.9 (d) | 67.6 (d) |
| 20 | 1.95 (1H, m)<br>1.51 (1H, m) | 1.43 (1H, t, 11.8)<br>2.00 (1H, m) | 40.6 (t) | 40.7 (t) |
| 21 | | | 95.9 (s) | 99.7 (s) |
| 22 | 5.54 (1H, dd, 10.0, 2.6) | 1.65 (1H, m)<br>1.97 (1H, m) | 128.1 (d) | 41.1 (t) |
| 23 | 5.73 (1H, d, 10.0) | 3.76 (1H, m) | 136.1 (d) | 70.0 (d) |
| 24 | 2.28 (1H, m) | 1.64 (1H, m) | 30.1 (d) | 35.1 (d) |
| 25 | 3.32 (1H, d, 10.0) | 3.41 (1H, m) | 77.3 (d) | 72.5 (d) |
| 26 | 1.54 (1H, m) | 1.51 (1H, m) | 38.7 (d) | 38.1 (d) |
| 27 | 1.78 (1H, m)<br>1.21 (1H, m) | 1.81 (1H, m)<br>1.23 (1H, m) | 27.0 (t) | 26.9 (t) |
| 28 | 1.78 (1H, m)<br>1.21 (1H, m) | 1.81 (1H, m)<br>1.21 (1H, m) | 26.5 (t) | 26.5 (t) |
| 29 | 1.54 (2H, m) | 1.51 (2H, m) | 31.4 (t) | 31.7 (t) |
| 30 | 1.64 (2H, m) | 1.61 (1H, m)<br>1.21 (1H, m) | 25.6 (t) | 24.5 (t) |
| 31 | 1.26 (1H, m)<br>1.64 (1H, m) | 1.21 (1H, m)<br>1.67 (1H, m) | 26.6 (t) | 26.5 (t) |
| 4-Me | 2.24 (3H, s) | 1.82 (3H, br s) | 15.3 (q) | 19.9 (q) |
| 5-$OCH_3$ | | 3.50 (3H, s) | | 57.8 (q) |
| 8-Me | 2.07 (3H, br s) | | 18.2 (q) | |
| 12-Me | 1.17 (3H, d, 6.9) | 1.16 (3H, d, 6.9) | 19.6 (q) | 20.3 (q) |
| 14-Me | 1.57 (3H, br s) | 1.51 (3H, br s) | 15.6 (q) | 15.2 (q) |
| 24-Me | 0.92 (3H, d, 7.2) | 0.91 (3H, d, 6.8) | 16.6 (q) | 13.8 (q) |
| 1′ | 4.83 (1H, d, 3.5) | 4.81 (1H, d, 3.4) | 95.0 (d) | 95.0 (d) |
| 2′ | 1.54 (1H, m)<br>2.39 (1H, m) | 1.52 (1H, m)<br>2.27 (1H, m) | 33.9 (t) | 31.2 (t) |
| 3′ | 3.60 (1H, m) | 3.55 (1H, m) | 78.3 (d) | 78.3 (d) |
| 3′-$OCH_3$ | 3.53 (3H, s) | 3.47 (3H, s) | 56.8 (q) | 56.6 (q) |
| 4′ | 3.18 (1H, t, 9.1) | 3.17 (1H, t, 9.0) | 76.2 (d) | 76.1 (d) |
| 5′ | 3.89 (1H, dd, 9.1, 6.2) | 3.76 (1H, m) | 67.9 (d) | 68.1 (d) |
| 5′-Me | 1.28 (3H, d, 6.2) | 1.28 (3H, d, 6.0) | 17.7 (q) | 17.7 (q) |

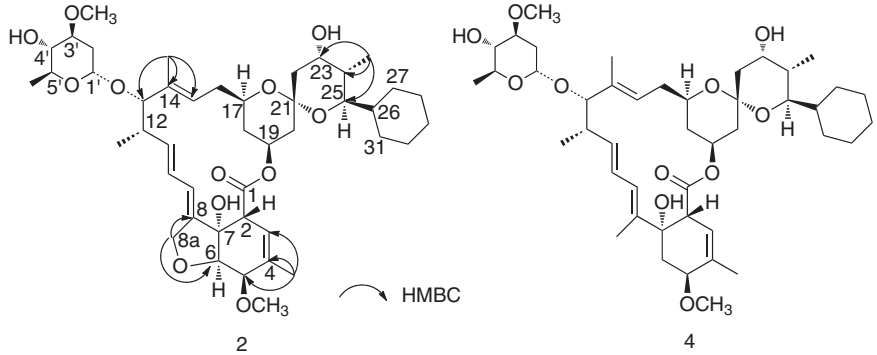


**Figure 2** Structures of **2**, **4** and key HMBC correlations of **2**.

was also an analog of selamectin. The difference between **2** and **4** is that compound **2** has the furan ring moiety as that of selamectin, which is supported by the 14 mass unit difference from **4**. This conclusion was further supported by the HMBC correlation from 8a-$H_2$ ($\delta_H$ 4.63/4.70) to C-6 ($\delta_C$ 77.4) and C-8 ($\delta_C$ 139.8) (Figure 2). The relative configuration of **2** was also assigned by the analogy with **4** and selamectin.

Avermectins have attracted extensive attention due to the impressive anthelmintic and insecticidal activity. Until now, six avermectins including abamectin, doramectin, aprinomectin, ivermectin and selamectin have been successfully commercialized, and they are considered to be the most widely used drugs in animal health and agriculture.[4–6] Similar to selamectin that exhibits high insecticidal activities,[8] compounds **1** and **2** possess a monosaccharide subunit attached at C13. Therefore, the bioactivities of **1** and **2** were evaluated. Unfortunately, compounds **1** and **2** exhibited weak acaricidal and insecticidal activities even at a concentration of 100 $\mu g\,ml^{-1}$.

In conclusion, two new doramectin analogs were obtained from the culture broth of the mutant *S. avermitilis* NEAU1069-3. Along with previously obtained analogs of doramectin, avermectin and milbemycin from *S. avermitilis* NEAU1069, the discovery of compounds **1** and **2** may have important roles in understanding and perfecting the proposed biosynthetic pathways of doramectins.

## ACKNOWLEDGEMENTS

This research was supported by NNSF (no. 31071750) of China, NKPBR (no. 2010CB126102), the National Key Technology R&D Program (no. 2012BAD19B06), the National Outstanding Youth Foundation (no. 31225024), and the Program for New Century Excellent Talents in University (NCET-11-0953).

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