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ARTICLE

Isolation and Identification of Novel Macrocyclic Lactones from *Streptomyces avermitilis* NEAU1069 with Acaricidal and Nematocidal Activity†

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Bioactivity-guided fractionation of *Streptomyces avermitilis* NEAU1069 fermentation broth was used to isolate and determine the chemical identity of bioactive constituents with acaricidal and nemotocidal activity. The structures of novel compounds **1** and **2** were determined on the basis of spectroscopic analysis, including 1D and 2D NMR as well as HRESI-MS, ESI-MS of spectrometry analysis, UV and IR spectroscopic analyses, and comparison with data from the literature. The acaricidal activities of the isolated compounds against adult mites and mite eggs were evaluated by mortality and unhatched eggs. The nematocidal activity of the isolated compounds against *Caenorhabditis elegans* was calculated according to the immobilized rates against the total number of tested nematodes. The results indicated that compounds **1** and **2** exhibited potent acaricidal activity against adult mites, with a mortality of >90% at a concentration of 30 μg/mL. However, compounds **1** and **2** showed only weak acaricidal activity against mite eggs, with unhatched mite egg rates of <60% at a concentration of 100 μg/mL. Compound **2**, a hydroxylated derivative at C-23 of **1**, possessed a high nematocidal activity against *C. elegans*, with an immobility of >90% at a concentration of 10 μg/mL. These results demonstrate that compounds **1** and **2**, especially compound **2**, have potential as pesticides with acaricidal and nematocidal activity.

KEYWORDS: *Streptomyces avermitilis* NEAU1069; novel macrolide compounds; acaricidal activity; nematocidal activity

INTRODUCTION

The tremendous increase in crop yields associated with the "green" revolution has been possible in part by the discovery and utilization of chemicals for pest control. However, concerns over the potential impact of pesticides on human health and the environment have led to the introduction of new pesticide registration procedures, such as the Food Quality Protection Act in the United States. These new regulations have reduced the number of synthetic pesticides available in agriculture. Therefore, the current paradigm of relying almost exclusively on chemicals for pest control may need to be reconsidered. New pesticides, including natural product-based pesticides, are being discovered and developed to replace the compounds lost due to the new registration requirements (*1*−*3*).

Microbial metabolites attract increasing attention as potential pesticides. They are expected to overcome the resistance and pollution that have accompanied the use of synthetic pesticides (*4*, *5*). Several microbial metabolites, such as the avermectin and milbemycin families, have proven to be potent preventatives against a variety of pests such as insects and parasites. Moreover, they are the biggest selling and arguably most effective acaricides and anthelmintics currently available (*6*−*8*). In addition, blasticidin, polyoxin, kasugamycin, validamycin, and mildiomycin as fungicides, bialaphos as herbicide, and spinosad as insecticide have been widely used (*3*). The excellent activity of these compounds suggests that other desirable pesticides will be discovered from microbial metabolites (*1*, *2*, *9*, *10*).

We conducted our experiments to screen for new antibiotics for pesticides and antiparasitic veterinary drugs or as semisynthetic intermediates. As a result, two novel macrolide compounds (compounds **1** and **2**, **Figure 1**) were isolated from the fermentation broth of *Streptomyces avermitilis* NEAU1069 strain, which was newly obtained from a soil sample. Compounds **1** and **2** are reminiscent of doramectin, milbemycins (**Figure 1**), milbemycins β_3 (*11*−*14*), β_4 (*15*, *16*), β_{13} (*17*), and β_{14} (*17*), and aromatic S541 analogues (*18*). Doramectin and the two novel compounds differ in several aspects including the lack of the disaccharide group at C-13. In addition, doramectin possesses a hexahydrobenzofuran ring, whereas in compounds **1** and **2** this fused ring system is missing and instead an aromatic ring is present. The aromatic ring of the two novel compounds is the same as found in milbemycins β_3 (*11*−*14*), β_4 (*15*, *16*), β_{13} (*17*), and β_{14} (*17*) and

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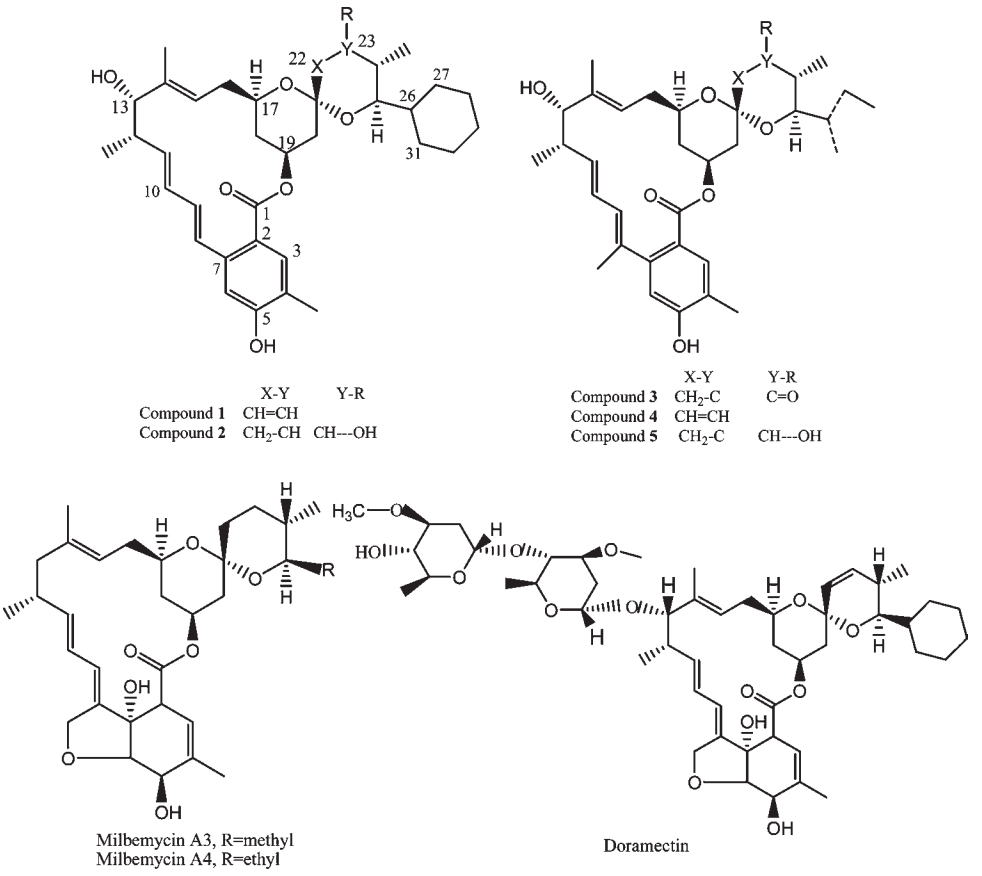

Figure 1. Structures of compounds **1**−**5**, milbemycins A_3 and A_4, and doramectin.

aromatic S541 analogues (*18*). The two new compounds also have a cyclohexyl group attached to C-25 as observed in doramectin. Moreover, there is no report about a C-22−C-23 double bond presence in milbemycins. The discovery of these novel compounds possibly plays an important role in the creation of new pesticides and in understanding and perfecting the proposed biosynthetic pathways of avermectins and milbemycins. This paper describes the fermentation, isolation, identification, and acaricidal and nematocidal activities of compounds **1** and **2**.

MATERIALS AND METHODS

General Procedures and Reagents. Melting points were measured with a Fisher-Johns micromelting point apparatus. UV spectra were obtained on a Varian Cary 300 BIO spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer; ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. Chemical shifts are reported as parts per million (δ), using the residual $CHCl_3$ (δ_H 7.26; δ_C 77.0) as an internal standard, and coupling constants (J) in hertz. ^{1}H and ^{13}C NMR assignments were supported by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. The ESI-MS and HRESI-MS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer. Optical rotation was measured on a Perkin-Elmer 341 polarimeter. Column chromatography was carried out on silica gel (100−200 mesh, Qing Dao Hai Yang Chemical Group Co., China). Semipreparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm i.d.; Agilent) was further performed to obtain pure compounds. All chemicals used in the study, such as methanol (MeOH), ethyl acetate (EtOAc), petroleum ether (60−90 °C), and acetone, were of analytical grade.

Microorganism. The producing organism, *S. avermitilis* NEAU1069, was isolated from a soil sample collected in Harbin, China. *S. avermitilis* NEAU1069 has been deposited at the China General Microbiology Culture Collection Center (accession no. CGMCC 2943), Institute of Microbiology, Chinese Academy of Sciences. The 16S rDNA sequence (accession no. DQ768097 in GenBank, National Center for Biological Information) was determined.

Fermentation. The strain was maintained on yeast extract−malt extract−soluble starch (YMS) medium containing 10 g of soluble starch, 2 g of yeast extract, 1 g of KNO_3, and 20 g of agar in 1.0 L of tap water, pH 7.0. The seed medium consisted of 20 g of glucose, 15 g of soybean flour, and 5.0 g of yeast autolysate in 1.0 L of water, pH 7.0. All of the media were sterilized at 121 °C for 20 min. Slant culture was incubated for 6−8 days at 28 °C. Ten milliliters of sterile water was added to the slant of YMS medium. The spores were scraped and transferred to a sterile screw-cap glass tube and shaken vigorously to break spore clumps. The spore suspension was then filtered through six layers of sterile filter cheesecloth and adjusted to 10^7−10^8 cfu/mL. Two milliliters of the spore suspension was inoculated into a 250 mL flask containing 25 mL of seed medium and incubated at 28 °C for 24 h at 250 rpm. Then 8.0 mL of the culture was transferred into a 1 L Erlenmeyer flask containing 100 mL of the producing medium, which consisted of 10% corn starch, 1% soybean powder, 1% cotton flour, 0.02% α-amylase, 0.1% NaCl, 0.2% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.7% $CaCO_3$, and 0.1% cyclohexanecarboxylic acid, pH 7.0, before sterilization. Fermentation was carried out at 28 °C for 12−13 days on a rotary shaker at 250 rpm.

Isolation and Purification of Compounds 1 and 2. Three liters of broth from 40 producing fermentations was filtered. The resulting cake was washed with water (3 L), and both filtrate and wash were discarded. Methanol (1 L) was used to extract the washed cake. The MeOH extract was evaporated under reduced pressure to approximately 0.2 L at 45 °C, and the resulting concentrate was extracted three times with an equal volume of EtOAc. The combined EtOAc phase was concentrated under reduced pressure to yield 5 g of oily substances. The residual oily substance was subjected to a silica gel column chromatography (CC) and eluted with a mixture of petroleum ether/acetone of increasing polarity to afford three fractions. Fraction III eluted with a mixture of petroleum ether/acetone (75:25, v/v) had the highest acaricidal activity against adult mites. This was further separated over silica gel CC eluted with petroleum/EtOAc (95:5, 85:15, and 75:25, v/v) to give three subfractions. Subfraction II eluted with

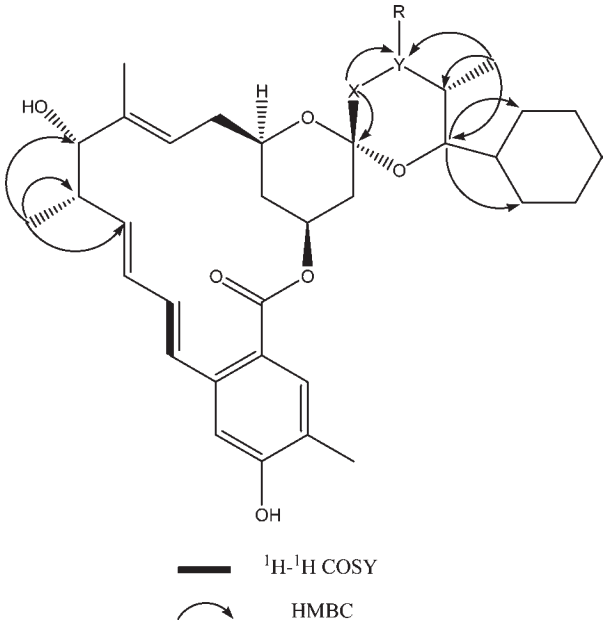

Figure 2. Key ^{1}H−^{1}H COSY and HMBC correlations of compounds **1** and **2**.

a mixture of petroleum/EtOAc (85:15 v/v) with acaricidal activity against adult mites, and it was separated by semipreparative HPLC eluting with CH_3OH/H_2O (92:8, v/v) to afford compound **1** (**Figure 1**) (t_R = 18.9 min, 14 mg). Subfraction III was eluted with a mixture of petroleum/EtOAc (75:25, v/v) and had the highest acaricidal activity against adult mites. This was purified by semipreparative HPLC using CH_3OH/H_2O (92:8, v/v) to obtain compound **2** (**Figure 2**) (t_R = 14.0 min, 12 mg).

Compound **1** ($C_{35}H_{46}O_6$) was obtained as a white amorphous powder: mp 143−145 °C; $[\alpha]_D^{25}$ +124° (*c* 0.088, EtOH); UV (EtOH) λ_{max} nm (log ε) 200 (4.83), 249 (4.57), 283 (4.25); IR (KBr), ν_{max} cm^{-1} 3417, 2927, 2854, 1706, 1610, 1504, 1450, 1365, 1278, 1157, 994; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in **Table 1**; ESI-MS *m*/*z* 563 $[M + H]^+$; HRESIMS *m*/*z* 585.3102 $[M + Na]^+$, calcd for $C_{35}H_{46}O_6Na$ 585.3187.

Compound **2** ($C_{35}H_{48}O_7$) was obtained as a white amorphous powder: mp 162−165 °C; $[\alpha]_D^{25}$ +66° (*c* 0.104, EtOH); UV (EtOH) λ_{max} nm (log ε) 200 (5.02), 250 (4.63), 282 (4.33); IR (KBr), ν_{max} cm^{-1} 3445, 2928, 2855, 1699, 1615, 1452, 1382, 1278, 1163, 994; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in **Table 1**; ESI-MS *m*/*z* 581 $[M+H]^+$; HRESIMS *m*/*z* 603.3292 $[M+Na]^+$, calcd for $C_{35}H_{48}O_7Na$ 603.3292.

Acaricidal Activity against Adult Mites. MeOH solutions containing 0.1% of the individual compounds were diluted 10-fold with water containing 0.01% of detergent to prepare 100 μg/mL solutions. Then appropriate further dilutions were prepared. Two-spotted spider mites, sensitive to organophosphorus insecticides, were inoculated on the primary leaves of cowpea plants. One day after inoculation, leaves of cowpea plants were soaked in the sample solutions for 1−2 s, and the leaves were kept at 25 °C. After 3 days, survival of the adult insects was determined with a binocular microscope, and the mortality (%) was calculated.

Acaricidal Activity against Mite Eggs. Sample solutions containing 100, 50, 30, and 10 μg/mL of individual compounds were prepared. Female adult two-spotted spider mites were allowed to lay eggs on the primary leaves of cowpea plants. The adult mites were removed to obtain test leaves each bearing about 40 eggs. In a similar manner to the preceding example, the test leaves were soaked in the sample solutions for 1−2 s. After 10 days at 25 °C, the number of unhatched eggs was counted, and the unhatched egg rates (%) were calculated.

Nematocidal Activity. MeOH solutions containing 0.1% of individual compounds were diluted 10-fold with water to prepare solutions containing 100 μg/mL. Then appropriate amounts of the solutions were added to 1 mL portions of an aqueous suspension containing living nematodes *Caenorhabditis elegans*. The mixtures were left at 25 °C for 15 h after shaking. The number of nematodes that were immobilized and the total number of the nematodes tested were counted under a stereoscopic microscope. Immobilized rates (%) against the total number of tested nematodes were calculated.

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (Coupling Constants in Parentheses)

	proton		carbon	
position	**1**	**2**	**1**	**2**
1			169.6 s[a]	169.6 s
2			123.6 s	123.6 s
3	7.36 s	7.37 s	132.5 d	132.6 d
4			122.7 s	122.9 s
5			155.6 s	155.9 s
6	6.56 s	6.56 s	115.3 d	115.3 d
7			137.1 s	137.1 s
8	6.45 d (15.1)	6.45 d (14.9)	128.7 d	128.8 d
9	6.09 dd (10.4, 15.1)	6.06 dd (10.4, 14.9)	134.4 d	134.3 d
10	6.01 dd (10.4, 14.5)	6.01 dd (10.4, 14.4)	130.4 d	130.4 d
11	5.62 dd (9.9, 14.5)	5.61 dd (9.8, 14.4)	135.7 d	135.7 d
12	2.58 m	2.58 m	40.6 d	40.6 d
13	4.04 br s	4.04 br s	78.5 d	78.3 d
14			138.1 s	138.6 s
15	5.29 br d (10.8)	5.28 m	117.7 d	117.0 d
16	2.31 m	2.31 m	33.8 t	33.6 t
17	3.99 m	3.79 m	68.6 d	68.6 d
18	0.88 q (12.1)	0.88 q (12.0)	36.5 t	36.2 t
	1.98 m	1.96 m		
19	5.56 m	5.48 m	68.1 d	67.3 d
20	1.50 t (11.8)	1.44 t (11.8)	40.8 t	41.1 t
	2.02 m	1.97 m		
21			95.9 s	99.9 s
22	5.53 dd (2.4, 10.0)	1.65 m	128.0 d	41.0 d
		1.97 m		
23	5.72 dd (1.2, 10.0)	3.76 m	136.0 d	70.3 d
24	2.29 m	1.65 m	30.1 d	35.1 d
25	3.32 d (10.0)	3.41 d (10.7)	77.3 d	72.5 d
26	1.53 m	1.49 m	38.7 d	38.1 d
27	1.63 m	1.58 m	25.6 t	24.5 t
	1.19−1.37 m	1.19−1.30 m		
28	1.19−1.37 m	1.19−1.30 m	26.7 t	26.6 t
	1.79−1.89 m	1.77−1.87 m		
29	1.65 m	1.67 m	26.5 t	26.5 t
30	1.19−1.37 m	1.19−1.30 m	27.0 t	26.9 t
	1.79−1.89 m	1.77−1.87 m		
31	1.53 m	1.50 m	31.6 t	31.2 t
	1.63 m	1.63 m		
4a	2.21 s	2.21 s	15.3 q	15.4 q
12a	1.22 d (6.9)	1.20 d (6.9)	18.4 q	18.5 q
14a	1.58 br s	1.58 br s	14.8 q	14.8 q
24a	0.92 d (7.1)	0.92 d (6.9)	16.6 q	13.6 q

[a] By DEPT sequence.

RESULTS AND DISCUSSION

Structural Elucidation of Compounds 1 and 2. Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{35}H_{46}O_6$ on the basis of HRESIMS at *m*/*z* 585.3102 $[M + Na]^+$ (calcd 585.3187 for $C_{35}H_{46}O_6Na$) and ^{13}C NMR data (**Table 1**). The IR spectrum of compound **1** showed a hydroxyl and one ester carbonyl absorption at 3445 and 1699 cm^{-1}, respectively. The ^{1}H NMR spectrum of compound **1** indicated two aliphatic methyl doublets at δ 0.92 and 1.20, two olefinic or aromatic methyl signals at δ 1.58 and 2.21, and two downfield singlet proton signals at δ 7.36 and 6.56. Its ^{13}C NMR spectrum displayed 35 carbon signals, including 1 ester carbonyl, 14 sp^2 carbons, 4 methyls, 8 methylenes, 7 aliphatic methines (including four oxygenated ones), and 1 ketal carbon. The ^{1}H and ^{13}C NMR data were compared with those of doramectin.

The spectra in conjunction with the same producing strain as doramectin suggested that compound **1** may be a derivative of doramectin aglycone. Detailed comparison of the NMR data of compound **1** with those of milbemycins β_3 (*13*, *14*), β_{13}, and β_{14} (*17*) isolated from the milbemycin-producing strain revealed that compound **1** was similar to milbemycin β_3 except the differences of C-8, C-13, C-23, and C-25 positions. In the HMBC spectrum, the observed correlation between δ_H 1.20 and δ_C 135.7, 40.6, and 78.5 showed that the C-13 was substituted by a hydroxyl group. The observed correlation of δ 5.53 and 5.72 in $^1H-^1H$ COSY spectrum and the HMBC correlated signal of δ_H 0.92 and δ_C 136.0 indicated that a double bond was located between C-22 and C-23. In addition to the downfield proton signals of C-22 and C-23, the olefinic proton signal of C-9 with two large coupling constants (J = 10.4, 15.1 Hz) and one additional olefinic proton at δ 6.45 comparable to those of milbemycins β_3 (*13*, *14*), β_{13}, and β_{14} (*17*) showed that the vinyllic methyl of C-8a in compound **1** had disappeared. The left five aliphatic methylenes and one aliphatic methine in conjunction with one unsaturation showed the presence of a cyclohexyl group. The three bond correlation signals between δ_H 3.32 and δ_C 31.6 and 25.6 assigned the cyclohexyl group at C-25 as that of doramectin. Therefore, the structure of compound **1** was elucidated as shown in **Figure 1**. The relative stereochemistry of compound **1** was assigned as occurring with that of doramectin (*19*).

Compound **2** was obtained as a white amorphous powder. Its ^1H and ^{13}C NMR data were very similar to those of compound **1**, except for the absence of a double bond and the presence of one methine bonding with oxygen and one methylene. These data in conjunction with the HMBC correlations between δ_H 0.92 and δ_C 70.3 indicated that the C-22−C-23 double bond in compound **1** was replaced by one hydroxymethine on C-23 and a methylene for C-22 in compound **2**. The relative configuration of compound **1** was assigned on the basis of the concurrence with that of 25-cyclohexyl-avermectin B_2 (*19*). Thus, the structure of compound **2** was established.

We have determined the 16S rDNA sequence of the strain (DQ768097), which produces compounds **1** and **2**. This shared 99.05, 99.93, and 99.93% identity with the 16S rDNA sequences of *S. avermitilis* strains NBRC 14893 (AB184632), MA-4680 (AB078897), and AF145223, respectively, thereby characterizing strain NEAU1069 as a *S. avermitilis*. We also isolated avermectin B1a and compounds **3**, **4**, and **5** (**Figure 1**) (*20*) from the *S. avermitilis* NEAU1069 fermentation broth. Furthermore, compounds **3**−**5** produced by *S. avermitilis* NEAU1069 had the same isopropyl group at C-25 as avermectin. Although compounds **3**−**5** are analogues of milbemycins β_{13} and β_{14} (*17*) produced by *Streptomyces bingchenggensis*, the 16S rDNA sequence of *S. avermitilis* NEAU1069 shared only 94.84% identity compared with that of *S. bingchenggensis* (DQ449953). To our knowledge, the β-class milbemycin compounds isolated from *S. avermitilis* are reported for the first time in this study.

Doramectin could be obtained by *S. avermitilis* with the addition of cyclohexanecarboxylic acid to avermectin-producing medium (*21*−*23*). Therefore, we added cyclohexanecarboxylic acid to the producing medium for *S. avermitilis* NEAU1069. As we expected, novel compounds **1** and **2** with a cyclohexyl group at the position of C-25, like doramectin, were generated. This result demonstrated that *S. avermitilis* NEAU1069, similar to *S. avermitilis*, could biosynthesize novel analogues of compounds **3**−**5** (*23*) with different groups when various substances were put into the producing medium.

Acaricidal and Nematocidal Activity of the Two Novel Compounds 1 and 2. Compounds **1** and **2** from the *S. avermitilis* NEAU1069 fermentation broth were tested for their acaricidal and nematocidal activity. The acaricidal and nematocidal capacity of compounds was compared with those shown by the two known standard acaricides and nematocides, milbemycin A_3/A_4 and doramectin, in the same assay. As shown in **Table 2**, compounds **1** and **2** exhibited a potent acaricidal activity against adult mites, with a mortality of >90% at a concentration of 30 μg/mL. Their acaricidal activity against adult mites was similar to that of doramectin. However, compounds **1** and **2** showed only weak acaricidal activity against mite eggs, with unhatched mite egg rates of <60% at a concentration of 100 μg/mL (**Table 3**). The acaricidal activity against mite eggs was higher than that of doramectin, but lower than that of milbemycin. Compound **2**, the hydroxylated derivative at C-23, possessed a high nematocidal activity against *C. elegans*, with an immobility of >90% at a concentration of 10 μg/mL (**Table 4**); the nematocidal activity against *C. elegans* was similar to that of doramectin, but was higher than that of milbemycin.

Compounds **1** and **2** are avermectin and milbemycin analogues, respectively. Six avermectins (abamectin, doramectin, emamectin, eprinomectin, ivermectin, and selamectin) and four milbemycins (lepimectin, milbemectin, milbemycin oxime, and moxidectin) have been commercialized in crop protection and animal health. Moreover, new avermectin (*24*, *25*) and milbemycin derivatives (*26*, *27*) with extremely high insecticidal and

Table 2. Acaricidal Activity of Compounds **1** and **2**, Doramectin, and Milbemycin against Adult Mites[a]

	adult mite mortality (%)			
concn (μg/mL)	**1**	**2**	doramectin	milbemycin A_3/A_4[b]
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0
50	100 ± 0	100 ± 0	100 ± 0	100 ± 0
30	92.5 ± 3.3	96.4 ± 2.5	86.5 ± 2.0	95.3 ± 4.1
10	77.1 ± 6.3	75.6 ± 4.3	62.9 ± 6.4	73.7 ± 5.3
2	45.2 ± 5.7	43.9 ± 4.7	26.8 ± 6.1	39.3 ± 7.4
0	0	0	0	0

[a] Values are the means ± SDs of three independent experiments. [b] Milbemycins A_3 and A_4 mixtures, 30:70 (in volume).

Table 3. Acaricidal Activity of Compounds **1** and **2**, Doramectin, and Milbemycin against Mite Eggs[a]

	unhatched mite egg rates (%)			
concn (μg/mL)	**1**	**2**	doramectin	milbemycin A_3/A_4[b]
100	56.7 ± 3.6	59.8 ± 1.5	47.4 ± 1.9	71.3 ± 4.8
50	37.6 ± 4.2	43.7 ± 2.4	21.5 ± 1.7	49.7 ± 1.6
30	19.8 ± 2.4	23.5 ± 3.4	8.9 ± 3.5	35.9 ± 4.1
10	12.1 ± 1.7	13.9 ± 2.0	0	17.6 ± 2.2
2	0	0	0	5.6 ± 2.0
0	0	0	0	0

[a] Values are the means ± SDs of three independent experiments. [b] Milbemycins A_3 and A_4 mixtures, 30:70 (in volume).

Table 4. Nematocidal Activity of Compounds **1** and **2**, Doramectin, and Milbemycin against *Caenorhabditis elegans*[a]

	immobility (%)			
concn (μg/mL)	**1**	**2**	doramectin	milbemycin A_3/A_4[b]
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0
50	100 ± 0	100 ± 0	100 ± 0	100 ± 0
30	81.4 ± 6.1	100 ± 0	100 ± 0	88.6 ± 4.3
10	49.2 ± 1.9	92.7 ± 4.6	86.5 ± 5.4	51.4 ± 7.2
2	13.5 ± 3.8	43.7 ± 2.8	45.3 ± 4.1	21.7 ± 3.6
0	6.8 ± 1.2	5.9 ± 2.1	5.3 ± 1.5	5.4 ± 2.6

[a] Values are the means ± SDs of three independent experiments. [b] Milbemycins A_3 and A_4 mixtures, 30:70 (in volume).

acaricidal activity have been reported. Because this chemical class had different structures, they showed a greater diversity of functions (*28*, *29*). There are significant differences among the structures of compounds **1** and **2**, avermectins and milbemycins, which probably suggested that they performed various roles.

In conclusion, compounds **1** and **2** produced by *S. avermitilis* NEAU1069 possess acaricidal and nematocidal activity. Especially, compound **2** had not only higher acaricidal activity against adult mites but also higher nematocidal activity. Therefore, compound **2** has potential as a biologically based pesticide.

LITERATURE CITED

(1) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034.

(2) Copping, L. G.; Duke, S. O. Natural products that have been used commercially as crop protection agents. *Pest Manag. Sci.* **2007**, *63*, 524–554.

(3) Wheeler, W. B. Role of research and regulation in 50 years of pest management in agriculture. Prepared for the 50th anniversary of the *Journal of Agricultural and Food Chemistry*. *J. Agric. Food Chem.* **2002**, *50*, 4151–4155.

(4) Tanaka, Y.; Omura, S. Agroactive compounds of microbial origin. *Annu. Rev. Microbiol.* **1993**, *47*, 57–87.

(5) Saxena, S.; Pandey, A. K. Microbial metabolites as eco-friendly agrochemicals for the next millennium. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 395–403.

(6) Montesinos, E. Development, registration and commercialization of microbial pesticides for plant protection. *Int. Microbiol.* **2003**, *6*, 245–52.

(7) Fox, L. M. Ivermectin: uses and impact 20 years on. *Curr. Opin. Infect. Dis.* **2006**, *19*, 588–593.

(8) Ömura, S. Ivermectin: 25 years and still going strong. *Int. J. Antimicrob. Agents* **2008**, *31*, 91–98.

(9) Demain, A. L. A new era of exploitation of microbial metabolites. *Biochem. Soc. Symp.* **1983**, *48*, 117–132.

(10) Thompson, G. D.; Dutton, R.; Sparks, T. C. Spinosad − a case study: an example from a natural products discovery programme. *Pest Manag. Sci.* **2000**, *56*, 696–702.

(11) Davies, H. G.; Green, R. H. Avermectins and milbemycins, part I. *Chem. Soc. Rev.* **1991**, *20*, 211–269.

(12) Aoki, A.; Fukuda, R.; Nakayabu, T. Antibiotic substances. U.S. Patent 3,950,360, April 13, 1976.

(13) Smith, A. B.; Schow, S. R.; Bloom, J. D.; Thompson, A. S.; Winzenberg, K. N. Total synthesis of milbemycin β_3. *J. Am. Chem. Soc.* **1982**, *104*, 4015–4018.

(14) Schow, S. R.; Bloom, J. D.; Thompson, A. S.; Winzenberg, K. N.; Smith, A. B. Total synthesis of milbemycin β_3 and its C(12) epimer. *J. Am. Chem. Soc.* **1986**, *108*, 2662–2674.

(15) Nonaka, K.; Tsukiyama, T.; Okamoto, Y.; Sato, K.; Kumasaka, C.; Yammoto, T.; Maruyama, F.; Yoshikawa, H. New milbemycins from *Streptomyces hygroscopicus* subsp. *aureolacrimosus*: fermentation, isolation and structure elucidation. *J. Antibiot.* **2000**, *53*, 694–704.

(16) Hood, J. D.; Banks, R. M.; Brewer, M. D.; Fish, J. P.; Manger, B. R.; Poulton, M. E. A novel series of milbemycin antibiotics from *Streptomyces* strain E225. I. Discovery, fermentation and anthelmintic activity. *J. Antibiot.* **1989**, *42*, 1593–1598.

(17) Xiang, W. S.; Wang, J. D.; Wang, X. J.; Zhang, J. Two new β-class milbemycins from *Streptomyces bingchenggensis*: fermentation, isolation, structure elucidation and biological properties. *J. Antibiot.* **2007**, *60*, 351–356.

(18) Rudd, B. A. M. Macrolide compounds. E.P. 0,242,052, Oct 21, 1987.

(19) Dutton, C. J.; Gibson, S. P.; Kinns, M.; Swanson, A. G.; Bordner, J. Structure of doramectin. *J. Chem. Soc., Perkin Trans. 2* **1995**, 403–408.

(20) Wang, M.; Yang, X. H.; Wang, J. D.; Wang, X. J.; Chen, Z. J.; Xiang, W. S. New β-class milbemycin compound from *Streptomyces avermitilis* NEAU1069: fermentation, isolation and structure elucidation. *J. Antibiot.* **2009**, *62*, 587−591.

(21) Stutzman-Engwall, K.; Conlon, S.; Fedechko, R.; McArthur, H.; Pekrun, K.; Chen, Y.; Jenne, S.; La, C.; Trinh, N.; Kim, S.; Zhang, Y. X.; Fox, R.; Gustafsson, C.; Krebber, A. Semi-synthetic DNA shuffling of aveC leads to improved industrial scale production of doramectin by *Streptomyces avermitilis*. *Metab. Eng.* **2005**, *7*, 27–37.

(22) Stutzman-Engwall, K.; Conlon, S.; Fedechko, R.; Kaczmarek, F.; McArthur, H.; Krebber, A.; Chen, Y.; Minshull, J.; Raillard, S. A.; Gustafsson, C. Engineering the aveC gene to enhance the ratio of doramectin to its CHC-B2 analogue produced in *Streptomyces avermitilis*. *Biotechnol. Bioeng.* **2003**, *82*, 359–369.

(23) Dutton, C. J.; Gibson, S. P.; Goudie, A. C.; Holdom, K. S.; Pacey, M. S.; Ruddock, J. C.; Bu'Lock, J. D.; Richards, M. K. Novel avermectins produced by mutational biosynthesis. *J. Antibiot.* **1991**, *44*, 357–365.

(24) Pitterna, T.; Cassayre, J.; Hüter, O. F.; Jung, P.; Maienfisch, P.; Kessabi, F. M.; Quaranta, L.; Tobler, H. New ventures in the chemistry of avermectins. *Bioorg. Med. Chem.* **2009**, *17*, 4085–4095.

(25) Santos, A. R. D.; Falcão, C. A. B.; Muzitano, M. F.; Kaiser, C. R.; Rossi-Bergmann, B.; Férézou, J. P. Ivermectin-derived leishmanicidal compounds. *Bioorg. Med. Chem.* **2009**, *17*, 496–502.

(26) Xiang, W. S.; Wang, J. D.; Wang, X. J.; Zhang, J.; Wang, Z. Further new milbemycin antibiotics from *Streptomyces bingchenggensis*. *J. Antibiot.* **2007**, *60*, 608–613.

(27) Xiang, W. S.; Wang, J. D.; Wang, X. J.; Zhang, J. Two new milbemycins from *Streptomyces bingchenggensis:* fermentation, isolation, structure elucidation and biological properties. *J. Antibiot.* **2007**, *60*, 351–356.

(28) Wesley, L.; Shoop, H. M.; Michael, H. F. Structure and activity of avermectins and milbemycins in animal health. *Vet. Parasito.* **1995**, *59*, 139–156.

(29) McKellar, Q. A.; Benchaoui, H. A. Avermectins and milbemycins. *J. Vet. Pharmcol. Ther.* **1996**, *19*, 331–351.

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