ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:5 (2017) 431-438

records of natural products

New 5-oxomilbemycins from a Genetically Engineered Strain *Streptomyces bingchenggensis* BCJ60

Jiansong Li[1], Hui Zhang[3], Shaoyong Zhang[2], Haiyan wang[1], Ji Zhang[1], Anliang Chen[2], Jidong Wang[2,3*] and Wensheng Xiang[1*]

[1]*Life Science and Biotechnology Research Center, School of Life Science, Northeast Agricultural University, Harbin 150030, China*

[2]*Provincial Joint Engineering Laboratory of Biopesticide Preparation, School of Forestry & Biotechnology, Zhejiang Agricultural and Forestry University, Lin'An 311300, China*

[3]*Department of New Drug Screening, Zhejiang Hisun Pharmaceutical Co., Ltd, Taizhou 318000, China*

(Received November 21, 2016; Revised May 3, 2017; Accepted May 12, 2017)

Abstract: Two new 5-oxomilbemycins, 27-aldehyde-5-oxomilbemycin β_{12} (**1**) and 2-hydroxymilbemycin K (**2**), were isolated from a genetically engineered strain *Streptomyces bingchenggensis* BCJ60. Their structures were determined by comprehensive analyses of its ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC spectroscopic, HR-ESI-MS mass spectrometric and comparison with data from the literature. Preliminary studies showed that 27-aldehyde-5-oxomilbemycin β_{12} (**1**) and 2-hydroxymilbemycin K (**2**) possessed strong acaricidal and nematocidal activities.

Keywords: New 5-oxomilbemycins; genetically engineered strain; *Streptomyces bingchenggensis* BCJ60; acaricidal and nematocidal activities.

1. Introduction

Milbemycin oxime, 5-oxime derivate of milbemycins A3/A4, is a new semisynthetic macrolide veterinary drug and has been used as a broad-spectrum antiparasitic agent against worms, insects, and mites of pet animals in Japan, USA, Europe, and China [1-3]. Currently, milbemycin oxime is synthesized by a two-step chemical reaction, which involves the ketonization of milbemycins A3/A4 to yield the intermediates 5-oxomilbemycins A3/A4 with chromicanhydride as catalyst [4]. Due to the low efficiency and environmental unfriendliness of the ketonization of milbemycins A3/A4, it is imperative to develop alternative strategies to produce 5-oxomilbemycins A3/A4. In our earlier study, a genetically engineered strain *Streptomyces bingchenggensis* BCJ60 producing 5-oxomilbemycins as

* Corresponding authors: E-Mail: jdwang@hisunpharm.com; Phone:+86-576-88827216 Fax:+86-576-88828299
xiangwensheng@neau.edu.cn; Phone:+86-451-55190413 Fax:+86-451-55190413

The article was published by ACG Publications
www.acgpubs.org/RNP © Published 06/13/2017 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.55.16.11.563

main components was constructed by ARTP mutation system and site-specific genetic engineering cooperatively [5]. Previously, we have reported the isolation and structural elucidation of two new milbemycin derivatives from the genetically engineered strain *S. bingchenggensis* BCJ60 [6]. To further exploit the active constituents produced by this strain, the detailed fractionation of the crude extract was conducted and two new 5-oxomilbemycins, 27-aldehyde-5-oxomilbemycin β_{12} (**1**) and 2-hydroxymilbemycin K (**2**) (Figure 1) were subsequently obtained. In this paper, we describe the isolation, structural elucidation, acaricidal and nematocidal activities of compounds **1** and **2**.

2. Materials and Methods

2.1. General

Optical rotation was measured on a Perkin-Elmer 341 polarimeter (Anton Paar GmbH, Graz, Austria). UV spectra were recorded on a UV-1800 UV spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a Thermo Nicolet Avatar FT-IR spectrophotometer (Thermo, Tokyo, Japan) using KBr discs. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100MHz) spectra were measured with a Bruker DRX-400 (Agilent Technologies, Santa Clara, CA, USA) 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, HSQC, and HMBC experiments. The ESI-MS and HRESI-MS were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Agilent, Boblingen, Germany). Column chromatography (CC) was carried out on silica gel (100-200 mesh, Qingdao Haiyang Chemical Group Co., Qingdao, China). CC fractions were analyzed by TLC (silica gel GF_{254}, Qingdao Marine Chemical Factory, Qingdao, China). Semi-preparative HPLC (Agilent 1100, Zorbax SB-C3, 5 μm, 250×9.4 mm i.d.; Agilent, Palo Alto, CA, USA) was further performed to obtain pure compounds.

2.2. Fermentation and Isolation

The producing strain *S. bingchenggensis* BCJ60 was maintained on yeast extract-malt (YM) agar slant consisting of sucrose 0.4%, skim milk 0.1%, yeast extract (OXOID Basingstoke, Hampshire, UK) 0.2%, malt extract (BD Biosciences, San Jose, CA, USA) 0.5%, agar (BD Biosciences) 2.0% at 28°C for 6-8 days. A seed 15-L fermentor containing 6 L of seed medium (sucrose 1.0 %, polypepton 0.2 %, K_2HPO_4 0.05%, skim milk 0.05%) was inoculated with 0.5 L of broth cultured in flask with seed medium. The flask with seed medium (2-6×10^7 spores per ml) was cultured for 30 h at 28°C on a rotary shake at 250 r.p.m. After incubation for 30 h, the seed broth (6 L) in the 15-L fermentor was transferred into the production 100-L fermentor containing 60-L production medium (16.0% sucrose, 2.0% soybean powder, 0.5% yeast extract, 0.5% meat extract, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.005% $FeSO_4 \cdot 7H_2O$ and 0.3% $CaCO_3$). The culture temperature was 28°C and the initial pH was 7.40 sterilized by sparging with steam at 121°C for 30 min. The dissolved oxygen was maintained above 35% by adjusting the agitation speed. The initial aeration and agitation rate in the 15-L reactor was 1 vvm and 180 r.p.m., whereas those in the 100-L were 0.8 vvm and 150 r.p.m., respectively. The fermentation was performed for 10 days at 28°C.

The fermentation broth (60 L) was filtered. The resulting cake was washed with water, and both filtrate and wash were discarded. Methanol (20 L) was used to extract the washed cake. The MeOH extract was evaporated under reduced pressure to ~3 L at 50°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 40 g of oily substance. The residual oily substance was subjected to a silica gel column chromatography with a gradient petroleum ether/acetone solvent system (from 20:1 to 3:2) to give two fractions (I and II). Fraction II was further fractionated by silica gel eluted with petroleum ether/ethyl acetate (90:10, 85:15, and 80:20, v/v) to give three subfractions (A-C). Subfraction B was separated by semi-preparative HPLC eluting with $CH_3OH/CH_3CN/H_2O$ (42:42:16, v/v/v) to afford compounds **1** (R_t = 18.12 min, 13.3 mg) and **2** (R_t = 24.16 min, 14.5 mg).

*2.3. 27-aldehyde-5-oxomilbemycin β_{12} **(1)**.* White amorphous powder. $[\alpha]_D^{20}$ +102 (*c* 0.80, EtOH). UV (EtOH) λ_{max} nm (log ε): 288 (4.39), 227 (4.17), 200 (4.30). IR (KBr): 3449, 2927, 1733, 1669, 1451, 1334, 1178, 1057, 1037, 995, 909 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in Table 1. ESI-MS: *m/z* 525 $[M - H]^-$. HR-ESI-MS: *m/z* 527.2997 $[M + H]^+$ (calcd for $C_{31}H_{43}O_7$, 527.3003).

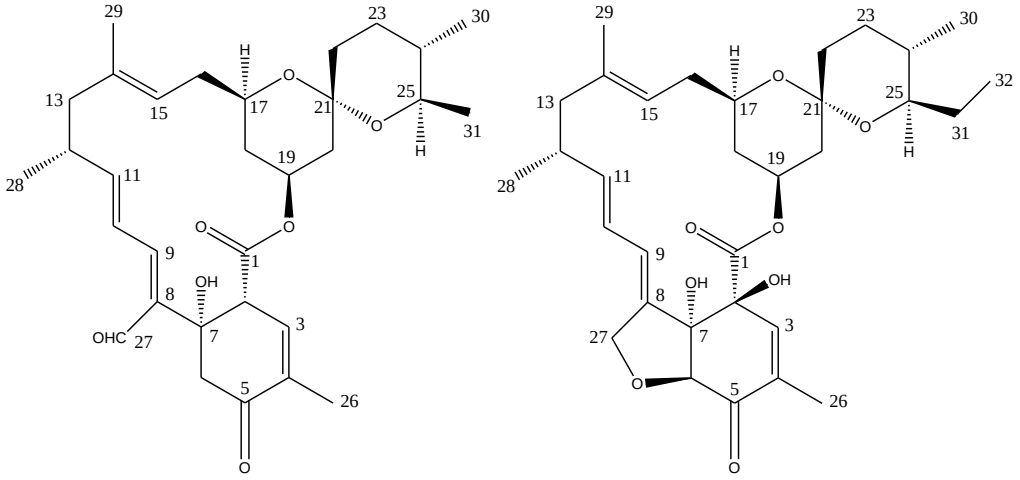
*2.4. 2-hydroxymilbemycin K **(2)**.* White amorphous powder. $[\alpha]_D^{20}$ +91 (*c* 0.40, EtOH). UV (EtOH) λ_{max} nm (log ε): 240 (4.38). IR (KBr): 3440, 2957, 2927, 1696, 1578, 1454, 1378, 1269, 1213, 1060, 988 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in Table 1. ESI-MS: *m/z* 557 $[M + H]^+$. HR-ESI-MS: *m/z* 574.3357 $[M + NH_4]^+$ (calcd for $C_{32}H_{48}NO_8$, 574.3374).

2.5. Acaricidal activity test

Each test sample was prepared in acetone at a concentration of 1,000 mg/L and diluted to the required concentrations of 0.01, 0.005, 0.0025, 0.001, and 0.0005 mg/L with distilled water containing alkylphenol ethoxylates (~1/1000, vol/vol). The primary leaves of *Vicia faba* L. species were infected with *T. cinnabarinus*. At 2 h after infection, 10 fourth instar mite larvae were dipped in the diluted solutions of related chemicals for 5 s before the superfluous liquid was removed, and the larvae were kept in a conditioned room. Three replicates were made for each concentration and a blank control. The mortality was evaluated 24 h after treatment by examining the adult mites under a binocular microscope to determine the living and dead individuals. The 50% lethal concentrations (LC_{50}s) of tested compounds were calculated using the probit method.

2.6. Nematicidal activity test

About 10 µl of each sample was added to an aqueous suspension (90 µl) containing approximately 2,500 living nematodes (third instar and fourth instar larvae of *Bursaphelenchus xylophilus*) per milliliter, and the suspension kept at 25°C for 24 h. The blank control group was prepared in the same way but lacked the tested compound. Three replicates were performed in each trial. Finally, the activities of the tested compounds were monitored under a microscope by recording the death rates of nematodes. Nematodes that did not move when prodded with a needle were considered to be dead. The LC_{50}s of tested compounds were calculated using the probit method.



1 **2**

Figure 1. Structures of compounds **1** and **2**.

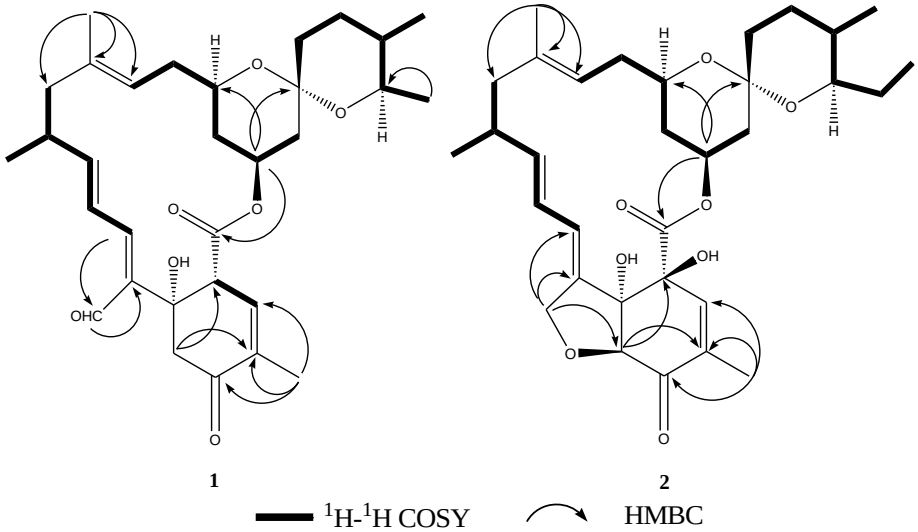


Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **2**.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was isolated as a white amorphous powder with a molecular formula $C_{31}H_{42}O_7$, as established by HR-ESI-MS at *m/z* 527.2997 $[M + H]^+$ (calcd for $C_{31}H_{43}O_7$, 527.3003), suggesting 11 degrees of unsaturation. The IR spectrum showed absorptions for hydroxyl group at 3449 cm^{-1} and carbonyl group at 1669 cm^{-1}. The ^{1}H NMR spectrum (Table 1) revealed five methyls at δ_H 0.82 (d, J = 6.5 Hz, H-30), 1.10 (d, J = 5.6 Hz, H-28), 1.12 (d, J = 6.0 Hz, H-31), 1.61 (s, H-29), and 1.86 (s, H-26); one trans-double bond at δ_H 5.86 (dd, J = 10.2, 14.2 Hz, H-11) and 6.79 (dd, J = 12.1, 14.2 Hz, H-10); one aldehyde at δ_H 10.22 (s, H-27). Detailed analysis of the NMR data indicated the presence of 31 carbon signals, which were classified as five methyls (δ_C 15.7, 16.0, 17.8, 19.3 and 21.2), seven methylenes (δ_C 27.7, 34.5, 35.6, 36.6, 40.7, 47.6 and 48.6), six aliphatic methines (δ_C 36.5, 36.6, 48.6, 67.2, 69.1 and 71.4), one oxygenated quaternary carbon (δ_C 75.7), one ketal carbon (δ_C 97.5), eight sp^2 carbons (δ_C 121.4, 121.5, 135.4, 136.2, 136.3, 136.7, 148.3, and 153.5), one aldehyde (δ_C 189.3), an ester carbonyl (δ_C 173.0), as well as one carbonyl group (δ_C 196.6). The ^{1}H-^{1}H COSY NMR spectrum (Figure 2) of **1** displayed a series of correlations: H-9 to H_3-28, H-15 to H_2-20, and H_2-22 to H_3-31. The HMBC correlations from H_3-26 (δ_H 1.86) to C-3 (δ_C 136.2), C-4 (δ_C 136.7) and C-5 (δ_C 196.6), from H_3-29 (δ_H 1.61) to C-13 (δ_C 48.1), C-14 (δ_C 136.3) and C-15 (δ_C 121.5), and from H-19 (δ_H 5.35) to C-1 (δ_C 173.0), C-17 (δ_C 67.2) and C-21 (δ_C 97.5) were observed. Analysis of the ^{1}H-^{1}H COSY, HSQC and HMBC spectra assigned the ^{1}H NMR and ^{13}C NMR data as shown in Table 1 and revealed compound **1** possessed the same macrocyclic lactone skeleton as 5-oxomilbemycin A3 (milbemycin J) [7-9] and milbemycin β_{12} [10]. Differences between compound **1** and 5-oxomilbemycin A3 were that the furan ring was missing and instead a C-27 aldehyde group was present in **1**. In addition, the C-6 oxygenated methine in 5-oxomilbemycin A3 was replaced by a methylene group in **1**. The differences between compound **1** and milbemycin β_{12} were that an aldehyde group and a carbonyl group were situated at C-27 and C-5, respectively, in **1**. The observed HMBC correlations from H-9 (δ_H 7.39) to C-27 (δ_C 189.3), from H-27 (10.22) to C-8 (δ_C 135.4), from H-6 (δ_H 2.40, 3.38) to C-2 (δ_C 48.6), C-4 (δ_C 136.7), and from H_3-26 (δ_H 1.86) to C-3 (δ_C 136.2), C-4 (δ_C 136.7) and C-5 (δ_C 196.6) supported the structural assignment of compound **1**. Therefore, the planar structure of compound **1** was elucidated and named 27-aldehyde-5-oxomilbemycin β_{12}. The relative configuration of **1** was assigned as occurring with 5-oxomilbemycins A3/A4.

Compound **2** had the molecular formula $C_{32}H_{44}O_8$ and 11 degrees of unsaturation, as deduced from HR-ESI-MS *m/z* 574.3357 $[M + NH_4]^+$ (calcd for $C_{32}H_{48}NO_8$, 574.3374). The IR absorption bands revealed the presence of hydroxyl (3440 cm^{-1}) and carbonyl (1696 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) indicated the presence of five methyls at δ_H 0.83 (d, J = 8.0 Hz, H-30), 1.03 (d, J = 6.4 Hz, H-28), 1.04 (t, J = 6.4 Hz, H-32), 1.55 (br s, H-29) and 1.97 (s, H-26), one trans-double bond at δ_H 5.50 (dd, J = 10.0, 14.5 Hz, H-11) and 5.82 (dd, J = 11.6, 14.5 Hz, H-10). The ^{13}C NMR (Table 1) and DEPT spectra showed 32 carbon signals, including five methyls (δ_C 10.1, 15.5, 15.7, 17.7, and 22.3), eight methylenes (δ_C 25.7, 27.8, 34.5, 35.5, 36.5, 41.3, 48.3, and 70.8), five aliphatic methines (δ_C 34.2, 35.7, 67.5, 71.1, and 76.1), three oxygenated quaternary carbons (δ_C 75.0, 81.7. 82.8), one ketal carbon (δ_C 97.5), eight sp^2 carbons (δ_C 121.0, 123.4, 123.6, 137.2, 137.3, 138.8, 140.3, and 144.7), an ester carbonyl (δ_C 171.4), and one carbonyl group (δ_C 194.2). The comparison of the spectral data of compound **2** and 5-oxomilbemycin A4 (milbemycin K) [7-9] showed a close similarity, except for the absence of a methine group (C-2 in 5-oxomilbemycin A4) in **2**. Instead, an oxygenated quaternary carbon (δ_C 75.0, C-2) was observed in **2**. Considered the molecular formula, a hydroxy group was situated at C-2. The HMBC correlations from H-6 to C-2 and C-4 further confirmed the assignment. Compound **2**, therefore, was established and named 2-hydroxymilbemycin K. The relative configuration of C-2-OH was attempted to assign as that of 2-hydroxy-3,4-dihydro-avermectin B_1 [11]. The other chiral centers of **2** were assigned as analogs to 5-oxomilbemycins A3/A4 [5].

3.2 Acaricidal and nematocidal capacities

The acaricidal and nematocidal capacities of compounds **1** and **2** were evaluated against *Tetranychus cinnabarinus* and *Bursaphelenchus xylophilus*, respectively [12-13]. The mixture of commercial acaricide and nematocide milbemycins A3/A4 was used as a positive control. As shown in Table 2, although compounds **1** and **2** exhibited strong acaricidal and nematocidal capacities, the bioactivities were weaker than those of milbemycins A3/A4, and this result will encourage us to further investigate the derivatives of milbemycin as the insecticidal agents in future.

Milbemycins have 16-membered macrolide structures, which are biosynthesized via a polyketide derived from the condensation of several units of acetate, propionate and branched-chain fatty acid [14]. The first reported members of the family, milbemycins, α_1 to α_{10} and β_1 to β_3 were isolated from *Streptomyces hygroscopicus subsp. aureolacrimosus* [15]. Subsequently, a further series of milbemycins (α_{11} to α_{27} and β_4 to β_{12}) were described from *Streptomyces cyaneogriseus subsp. noncyanogenus* and *Streptomyces thermoarchaensis* [16]. Apart from the mentioned strains, another newly milbemycin-producing strain *Streptomyces bingchenggensis* was isolated by our research team and currently used as an industrial producer of milbemycins [17]. *S. bingchenggensis* produces milbemycins A3/A4, two α-class milbemycins (B2/B3) and two β-class milbemycins (β_1/β_2) as its major components together with the polyether ionophore nanchangmycin and a trace of new milbemycin analogs, including α_{28}, α_{29}, α_{30}, β_{13}, β_{14}, ST906, and secomilbemycins A and B [18,19]. The α-class milbemycins with the furan ring at C-27 was generally associated with better acaricidal and nematocidal capacities than the β-class. Compounds **1** and **2** belong to β-class and α-class milbemycins, respectively. However, the nematocidal activity of compound **1** was superior to that of compound **2**. Maybe the aldehyde moiety at C-27 position was possible to improve the nematocidal activity.

Table 1. ^{1}H and ^{13}C NMR data (400 and 100 MHz, resp.) of compounds **1** and **2** in $CDCI_3$.

position	δ_H		δ_C	
	1	**2**	**1**	**2**
1			173.0	171.4
2	4.28 (1H, *br s*)		48.6	75.0
3	6.38 (1H, *br s*)	6.79 (1H, *s*)	136.2	140.3
4			136.7	137.3
5			196.6	194.2
6	2.40 (1H, *m*)	4.11 (1H, *s*)	47.6	82.8
	3.38 (1H, *m*)			
7			75.7	81.7
8			135.4	138.8
9	7.39 (1H, *d*, *J* = 12.1)	5.92 (1H, *d*, *J* = 11.6)	148.3	123.6
10	6.79 (1H, *dd*, *J* = 14.2, 12.1)	5.82 (1H, *dd*, *J* = 11.6, 14.5)	121.4	123.4
11	5.86 (1H, *dd*, *J* = 14.2, 10.2)	5.50 (1H, *dd*, *J* = 14.5, 10.0)	153.5	144.7
12	2.58 (1H, *m*)	2.48 (1H, *m*)	36.6	35.7
13	1.95 (1H, *m*)	1.92 (1H, *m*)	48.1	48.3
	2.23 (1H, *m*)	2.24 (1H, *m*)		
14			136.3	137.2
15	4.88 (1H, *br d*, *J* = 10.1)	5.01 (1H, *t*, *J* = 6.8)	121.5	121.0
16	2.24 (2H, *m*)	2.27 (2H, *m*)	34.5	34.5
17	3.57 (1H, *m*)	3.61 (1H, *m*)	67.2	67.5
18	1.69 (1H, *m*)	1.93 (1H, *m*)	36.6	36.5
	0.73 (1H, *m*)	1.05 (1H, *q*, *J* = 11.2)		
19	5.34 (1H, *m*)	5.59 (1H, *m*)	69.1	71.1
20	1.94 (1H, *m*)	2.05 (1H, *m*)	40.7	41.3
	1.39 (1H, *m*)	1.49 (1H, *t*, *J* = 11.8)		
21			97.5	97.5
22	1.53 (1H, *m*)	1.53 (1H, *m*)	35.6	35.5
	1.65 (1H, *m*)	1.70 (1H, *m*)		
23	1.53 (2H, *m*)	1.55 (2H, *m*)	27.7	27.8
24	1.26 (1H, *m*)	1.35 (1H, *m*)	36.5	34.2
25	3.23 (1H, *m*)	3.12 (1H, *m*)	71.4	76.1
26	1.86 (3H, *s*)	1.97 (3H, *s*)	15.7	15.7
27	10.22 (1H, *s*)	4.72 (2H, *s*)	189.3	70.8
28	1.10 (3H, *d*, *J* = 5.6)	1.03 (3H, *d*, *J* = 6.4)	21.2	22.3
29	1.61 (3H, *s*)	1.55 (3H, *s*)	16.0	15.5
30	0.82 (3H, *d*, *J* = 6.5)	0.83 (3H, *d*, *J* = 8.0)	17.8	17.7
31	1.12 (3H, *d*, *J* = 6.0)	1.39 (1H, *m*)	19.3	25.7
		1.73 (1H, *m*)		
32		1.04 (3H, *t*, *J* = 6.4)		10.1

Table 2. Acaricidal and nematocidal activities of compounds **1** and **2**.

Compounds	*Tetranychus cinnabarinus*		*Bursaphelenchus xylophilus*	
	LC_{50} (mg/L)[b]	*P*-value[c]	LC_{50} (mg/L)[b]	*P*-value[c]
1	0.197 ± 0.006	0.001	6.794 ± 1.058	0.215
2	0.168 ± 0.005	0.002	7.852 ± 0.116	0.001
milbemycins A3/A4[a]	0.107 ± 0.006	-	4.897 ± 0.042	-

[a] Milbemycins A3 and A4 mixtures, 30:70 (in volume).
[b] Values are the means ± SDs of three independent experiments.
[c] *P*-values are obtained by compared with milbemycins A3/A4 using Student's *t*-test.

Acknowledgments

This research was financially supported by grants from the National Outstanding Youth Foundation (No. 31225024), the National Natural Science Foundation of China (No. 31471832, 31171913, 31500010, 31572070 and 31372006), the National Key Technology R&D Program (No. 2012BAD19B06) and Chang Jiang Scholar Candidates Program for Provincial Universities in Heilongjiang (CSCP).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] S. E. Bienhoff, D. J. Kok, L. M. Roycroft and E. S. Roberts (2013). Efficacy of a single oral administration of milbemycin oxime against natural infections of Ancylostoma braziliense in dogs, *Vet. Parasitol*. **195**, 102-105.

[2] R. Schenker, D. Bowman, C. Epe, R. Cody, W. Seewald, G. Strehlau and P. Junquera (2007). Efficacy of a milbemycin oxime-praziquantel combination product against adult and immature stages of *Toxocara cati* in cats and kittens after induced infection, *Vet. Parasitol*. **145**, 90-93.

[3] J. W. McCall (**2005**). The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations, *Vet. Parasitol*. **133**, 197-206.

[4] Y. Tsukamoto, K. Sato, S. Mio, S. Sugai, T. Yanai, N. Kitano, S. Muramatsu and Y. Nakada, J. Ide (1991). Synthesis of 5-keto-5-oxime derivatives of milbemycins and their activities against microfilariae, *Agric. Biol. Chem.* **55**, 2615-2621.

[5] H. Y. Wang, J. Zhang, Y. J. Zhang, B. Zhang, C. X. Liu, H. R. He, X. J. Wang and X. S. Xiang (2014). Combined application of plasma mutagenesis and gene engineering leads to 5-oxomilbemycins A3/A4 as main components from *Streptomyces bingchenggensis*, *Appl. Microbiol. Biot.* **98**, 9703-9712.

[6] J. S. Li, M. N. Du, H. Zhang, J. Zhang, S. Y. Zhang, H. Y. wang, A. L. Chen, J. D. Wang and W. S. Xiang (2017). New milbemycin metabolites from a genetically engineered strain *Streptomyces bingchenggensis* BCJ60, *Nat. Prod. Res.* **31(7)**, 780-784.

[7] H. Mishima, J. Ide, S. Muramatsu, M. Ono (1983). Milbemycins, a new family of macrolide antibiotics. Structure determination of milbemycins D, E, F, G, H, J and K, *J. Antibiot.* **36**, 980-990.

[8] S. V. Ley, A. Madinb and N. J. T. Moncka (1993). Total synthesis of the spiroketal macrolide (+) milbemycin α_1, *Tetrahedron Lett.* **34**, 7479-7482.

[9] Y. Tsukamoto, K. Sato, S. Mio, S. Sugai, T. Yanai, N. Kitano, S. Muramatsu, Y. Nakada and J. Ide (1991) Synthesis of 5-keto-5-oxime derivatives of milbemycins and their activities against microfilariae, *Agric. Biol. Chem.* **55**, 2615-2621.

[10] N. Nonaka, T. Tsukiyama, Y. Okamoto, K. Kazuo Sato, C. Kumasaka, T. Yamamoto, F. Maruyama and H. Yoshikawa (2000). New Milbemycins from *Streptomyces hygroscopicus* subsp. aureolacrimosus: Fermentation, Isolation and Structure Elucidation, *J. Antibiot.* **73**, 694-704.

[11] J. Cassayre, T. Winkler, T. Pitterna and L. Quaranta (2010). Application of Mn(III)-catalysed olefin hydration reaction to the selective functionalisation of avermectin B1, *Tetrahedron Lett.* **51**, 1706-1709.

[12] J. Huang, A. L. Chen, H. Zhang, Z. Yu, M. H. Li, N. Li, J. T. Lin, H. Bai, J. D. Wang and Y. G. Zheng (2015). Gene replacement for the generation of designed novel avermectin derivatives with enhanced acaricidal and nematicidal activities, *Appl. Environ. Microb*. **81**, 5326-5334.

[13] J. J. Pan, X. Wan, H. Zhang, Z. Chen, J. Huang, B. Yang, A. L. Chen and J. D. Wang (2016). Three new milbemycins from a genetically engineered strain *S. avermitilis* MHJ1011, *J. Antibiot.* **69**, 104-107.

[14] K. Tanaka, H. Yoshikawa, M. Ando, S. Katsumi, K. Matsumoto, T. Toyama, M. Shibano and M. Abe (1993). Milbemycin: discovery and development, *Annu. Rep. Sankyo Res. Lab*. **45**, 1-98.

[15] Y. Takiguchi, H. Mishima, M. Okuda and M. Terao (1980). Milbemycin, a new family of macrolide antibiotics: fermentation, isolation and physicochemical properties, *J. Antibiot.* **33**, 1120–1127.

[16] J. D. Hood, R. M. Banks, M. D. Brewer, J. P. Fish, B. R. Manger and M. E. Poulton (1989). A novel series of milbemycin antibootics from Streptomyces strain E225. 1. Discovery, fermentation and anthelmintic activity, *J. Antibiot.* **42**, 1593–1598.

[17] X. J. Wang, X. C. Wang and W. S. Xiang (2009). Improvement of milbemycin producing *Streptomyces bingchenggensis* by rational screening of ultraviolet-and chemically induced mutants, *World J. Microbiol. Biotechnol.* **25**, 1051-1056.

[18] J. Zhang, J. An, J. J. Wang, Y. J. Yan, H. R. He, X. J. Wang and W. S. Xiang (2013). Genetic engineering of *Streptomyces bingchenggensis* to produce milbemycins A3/A4 as main components and eliminate the biosynthesis of nanchangmycin, *Appl. Microbiol. Biotechnol.* **97**, 10091-10101.

[19] W. S. Xiang, J. D. Wang, X. J. Wang and J. Zhang (2009), A novel macrolide compound from *Streptomyces bingchenggensis*: fermentation, isolation, structure elucidation and biological properties, *J. Antibiot.* **62**, 229-231.