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NOTE

New tenvermectin analogs obtained by microbial conversion with *Saccharopolyspora erythraea*

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Microbial transformation has been considered a powerful tool to modify the structures of natural bioactive substrates owing to its generally highly regio and stereo selective, simple, safe, environment friendly and relatively mild reaction conditions.[1–3] A wide variety of new derivatives of natural products can be obtained by oxidation,[4] hydrolysis,[5] isomerization, hydroxylation, esterification,[6] phosphorylation[7] and glycosylation[8] employing different conversion systems. Tenvermectins A and B are a kind of 16-membered macrocyclic lactone antibiotics isolated from the fermentation broth of genetically engineered strain *Streptomyces avermitilis* MHJ1011 with potent insecticidal properties.[9,10] Aiming at obtaining new target derivatives with improved insecticidal activity and lower toxicity, we carried out the transformation study of tenvermectins A and B by several microbial strains and no positive results were obtained. Inspired by literature,[11,12] we examined the microbial conversion of tenvermectins A and B by *Saccharopolyspora erythraea* ATCC 11635. This led to the isolation and identification of two new tenvermectin analogs, 4″-*O*-glucosyl tenvermectin A (**1**) and 4″-*O*-glucosyl tenvermectin B (**2**) (Figure 1). In this paper, we describe the microbial conversion of tenvermectins A and B, as well as the isolation, structural elucidation, nematocidal and acarcidal activity of the two new compounds.

The strain *S. erythraea*, purchased from the American Type Culture Collection (Manassas, VA, USA) under the accession number ATCC 11635, was maintained on a yeast-malt-starch (YMS) plate. Three 250 ml Erlenmeyer flasks, each containing 40 ml of seed medium consisted of peptone (Bei Jing Ao Bo Xing, Beijing, China) 0.3%, yeast extract (Bei Jing Ao Bo Xing) 0.2%, beef extract (Bei Jing Ao Bo Xing) 0.1% and glucose (Bei Jing Ao Bo Xing) 1%, pH 7.0, were inoculated with freshly obtained *S. erythraea* cultured from the YMS plate and cultivated on a rotary shaker with 250 r.p.m. at 28 °C for 40 h. Then, this culture was employed as an inoculum (5%, v/v) for sixty 250 ml Erlenmeyer flasks containing 50 ml of medium comprising dextrose (Bei Jing Ao Bo Xing) 4.4%, beef extract 0.5%, peptone 0.6%, yeast extract 0.4%, tryptone (Oxoid, Basingstoke, England) 0.3%, soybean flour (Ningbo Beilun Jiangnan Grease, Ningbo, China) 1.1%, NaCl 0.15% and K_2HPO_4 0.05% (pH adjusted to 7.2 prior to sterilization). All the media were sterilized at 121 °C for 20 min. The cultivation was performed on a rotary shaker at 28 °C with 250 r.p.m. After 24 h, the mixture of tenvermectins A and B solution (20 mg of tenvermectins A and B dissolved in 800 μl MeOH) was added to the growing culture and the incubation was continued for a further 120 h.

After a further 5-day incubation, the final 3 l of conversion broth was pooled and filtered. The mycelia cake was extracted with ethanol (1 l). The ethanol extract was then concentrated under

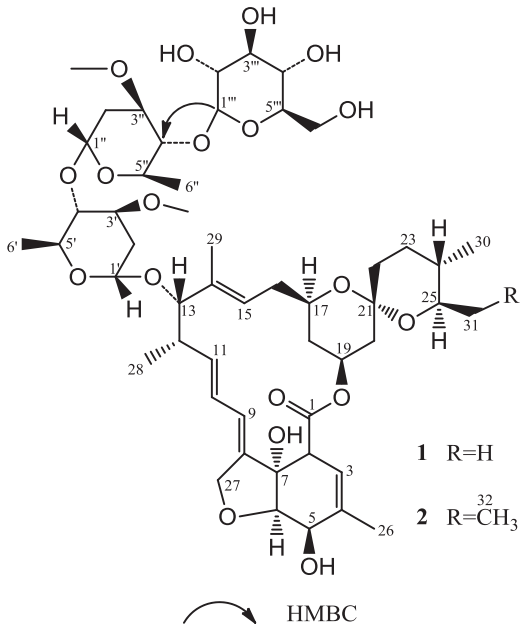

Figure 1 Structures and key HMBC correlations of compounds **1** and **2**.

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Table 1 Physicochemical properties of 1 and 2

	1	*2*
Appearance	White amorphous powder	White amorphous powder
$[\alpha]_D^{25}$ (EtOH)	−28.3 (c=1.8)	−30.9 (c=0.4)
MW	994	1008
Molecular formula	$C_{51}H_{78}O_{19}$	$C_{52}H_{80}O_{19}$
HRESI-MS (m/z)		
Calcd	1017.5030 $(M+Na)^+$	1031.5186 $(M+Na)^+$
Found	1017.5009 $(M+Na)^+$	1031.5161 $(M+Na)^+$
UV λ_{max}^{EtOH} (log ε)	245 (4.67), 239 (4.63)	245 (4.50), 239 (4.46)
IR ν_{max}^{KBr} (cm^{-1})	3387, 2930, 2875, 1721, 1646, 1450, 1383, 1340, 1305, 1270, 170, 1062, 989	3369, 2928, 2873, 1716, 1637, 1452, 1382, 1340, 1304, 1268, 1168, 1118, 1064, 985

reduced pressure to 150 ml at 45 °C and subsequently extracted three times using an equal volume of EtOAc. The combined organic layer was evaporated under reduced pressure to give 5 g of crude residue. The crude residue was applied on a silica-gel column (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–70:30, v/v) to afford two fractions (I and II) based on the TLC profiles. TLC was performed on silica-gel plates ($HSGF_{254}$, Yantai Chemical Industry Research Institute, Yantai, China), with solvent system of $CHCl_3$/MeOH (9:1). The developed TLC plates were observed under a UV lamp at 254 nm or by heating after spraying with sulfuric acid/ethanol, 5:95 (v/v). The fraction II (220 mg) eluted with $CHCl_3$/MeOH (85:15, v/v) was subjected to a Sephadex LH-20 gel (GE Healthcare, Glies, UK) column using $CHCl_3$/MeOH (50:50, v/v) as eluents and detected by TLC profiles to obtain two subfractions. The second subfraction was further isolated with semipreparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250×9.4 mm inner diameter; 1.5 ml min^{-1}; 245 nm; Agilent, Palo Alto, CA, USA) eluting with a MeOH/CH_3CN/H_2O mixture (45:45:10) to give compounds **1** (t_R 16.1 min, 28.6 mg) and **2** (t_R 19.9 min, 27.4 mg). Optical rotation values were measured on a Perkin-Elmer 341 polarimeter (Perkin-Elmer, Fremont, CA, USA). UV spectra were recorded on a Varian CARY 300 BIO spectrophotometer (Varian, Palo Alto, CA, USA), and the IR spectra were conducted on a Nicolet Magna FT-IR 750 spectrometer (Nicolet Magna, Madison, WI, USA); ^1H and ^{13}C NMR spectra were measured on a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The ESI-MS and HRESI-MS data were determined on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters, Milford, MA, USA).

The physicochemical properties of **1** and **2** are summarized in Table 1. The molecular formulas of **1** and **2** were determined to be $C_{51}H_{78}O_{19}$ and $C_{52}H_{80}O_{19}$, respectively, by HRESI-MS analysis, and their molecular masses were 162 mass units higher than the corresponding parent compounds, tenvermectins A and B, respectively. ^1H and ^{13}C NMR data for **1** and **2** were shown in Table 2. The NMR data of **1** and **2** were similar to those of tenvermectins A and B except of the additional sugar moiety revealed by the anomeric proton signals at δ_H 4.46 (1H, d, J=7.6 Hz) in **1** and at δ_H 4.46 (1H, d, J=7.5 Hz) in **2**. The chemical shift assignments and sugar linkage sites were determined by ^1H–^1H COSY, HMQC and HMBC experiments. By comparison of the chemical shifts and coupling constants of the additional sugar moiety in **1** and **2** with those of β-D-glucose residue

Table 2 ^1H and ^{13}C NMR data[a] of compounds 1 and 2

	δ_H (*J in Hz*)		δ_C	
Position	*1*	*2*	*1*	*2*
1			173.8 s	173.7 s
2	3.30 br s	3.29 br s	45.7 d	45.7 d
3	5.41 br s	5.41 br s	118.0 d	118.1 d
4			137.8 s	137.9 s
5	4.31 d (6.3)	4.31 d (6.2)	67.7 d	67.7 d
6	3.98 d (6.3)	3.97 d (6.2)	79.2 d	79.2 d
7			80.3 s	80.4 s
8			139.7 s	139.7 s
9	5.85 m	5.86 m	120.3 d	120.4 d
10	5.74 m	5.74 m	124.8 d	124.8 d
11	5.74 m	5.74 m	137.9 d	138.0 d
12	2.54 m	2.53 m	39.7 d	39.7 d
13	3.96 br s	3.95 br s	81.3 d	81.6 d
14			134.9 s	135.0 s
15	5.03 br d (9.7)	5.00 br d (10.2)	118.2 d	118.3 d
16	2.29 m	2.27 m	34.2 t	34.2 t
	2.35 m	2.34 m		
17	3.69 m	3.68 m	67.3 d	67.3 d
18	0.85 m	0.85 m	36.9 t	37.0 t
	1.80 m	1.79 m		
19	5.44 m	5.44 m	68.4 d	68.4 d
20	1.39 t (11.9)	1.39 t (12.1)	41.0	41.1
	2.00 dd (11.9, 4.3)	2.00 dd (12.1, 4.1)		
21			97.6 s	97.4 s
22	1.54 m	1.53 m	35.7 t	35.6 t
	1.69 m	1.68 m		
23	1.52 m	1.53 m	27.7 t	27.8 t
24	1.27 m	1.34 m	36.5 d	34.2 d
25	3.37 m	3.13 dd (9.8, 3.1)	71.4 d	75.9 d
26	1.89 br s	1.88 br s	19.9 q	19.9 q
27	4.67 dd (14.1, 2.1)	4.67 d (16.6)	68.4 t	68.5 t
	4.72 dd (14.1, 1.9)	4.71 d (16.6)		
28	1.17 d (6.0)	1.17 d (6.8)	20.3 q	20.3 q
29	1.52 br s	1.52 br s	15.2 q	15.2 q
30	0.85 d (6.6)	0.84 d (6.4)	17.9 q	17.7 q
31	1.17 d (6.0)	1.39 m	19.4 q	25.6 t
		1.71 m		
32		1.00 t (7.3)		10.0 q
1′	4.83 d (3.0)	4.81 d (2.8)	94.5 d	94.6 d
2′	2.28 m	2.27 m	34.7 t	34.6 t
	1.59 m	1.59 m		
3′	3.65 m	3.64 m	79.4 d	79.3 d
4′	3.24 t (9.0)	3.23 t (9.0)	80.6 d	80.6 d
5′	3.82 m	3.82 m	67.1 d	67.1 d
6′	1.25 d (6.1)	1.24 d (6.2)	18.4 q	18.4 q
1″	5.41 br s	5.40 br s	98.1 d	98.1 d
2″	1.53 m	1.53 m	34.5 t	34.5 t
	2.40 m	2.39 m		
3″	3.73 m	3.72 m	77.6 d	77.6 d
4″	3.26 m	3.25 m	85.5 d	85.6 d
5″	3.82 m	3.82 m	67.4 d	67.4 d
6″	1.33 d (6.2)	1.32 d (6.1)	17.6 q	17.6 q
1‴	4.46 d (7.6)	4.46 d (7.5)	105.4 d	105.5 d
2‴	3.39 m	3.38 m	75.1 d	75.1 d
3‴	3.59 m	3.57 m	76.3 d	76.3 d
4‴	3.59 m	3.57 m	70.1 d	70.2 d
5‴	3.39 m	3.38 m	75.8 d	75.8 d
6‴	3.89 m	3.87 m	62.3 t	62.5 t
	3.84 m	3.83 m		
3′-OMe	3.47 s	3.44 s	56.6 q	56.5 q
3″-OMe	3.44 s	3.43 s	56.0 q	56.0 q

[a]Chemical shifts are reported in p.p.m. (δ), using residual $CHCl_3$ (δ_H 7.26 p.p.m.; δ_C 77.0 p.p.m.) as an internal standard.

Table 3 Biological activity of compounds 1 and 2 against *B. xylophilus* and *T. cinnabarinus*

	LC_{50} (mg l^{-1})	
Compounds	*B. xylophilus*	*T. cinnabarinus*
1	6.7984	0.0156
2	5.7980	0.0113
Tenvermectin A	2.4391	0.0084
Tenvermectin B	1.3521	0.0059

that was contained in 2(*R*)-3-(4′-*O*-*β*-D-glucopyranosyl-3′-methoxyphenyl)propane-1,2-diol[13] and 6-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-5-*O*-*β*-D-glucopyranoside,[14] it was shown that the additional sugar moiety in **1** and **2** were *β*-D-glucose. The position of the glucosyl linkage in **1** and **2** were supported by the HMBC correlation (Figure 1) between the anomeric proton H-1‴ and C-4″. The assignment of the terminal glucose residue in **1** and **2** was further confirmed by the fact that the strain *S. erythraea* ATCC 11635 could transform the avermectin and ivermectin to the corresponding 4″-*O*-glucosyl derivatives.[12] Therefore, the structures of **1** and **2** (Figure 1) were identified as 4″-*O*-glucosyl tenvermectin A and 4″-*O*-glucosyl tenvermectin B, respectively.

The nematocidal and acaricidal activity of **1** and **2** against *Bursaphelenchus xylophilus* and *Tetranychus cinnabarinus*, respectively, reared in the laboratory were evaluated according to our reported paper.[9,10] In addition, for comparison, the nematocidal and acaricidal activity of their parent compounds, tenvermectins A and B, were examined. The bioassays were performed simultaneously on three replicates for each concentration. The bioassay results (Table 3) demonstrated that the two bioconversion products **1** and **2** showed almost the same or a slightly weaker nematocidal activity (LC_{50}s: **1**, 6.7984 mg l^{-1}; **2**, 5.7980 mg l^{-1}) than tenvermectins A and B against third-instar larvae of *B. xylophilus*. In addition, compounds **1** and **2** exhibited acaricidal activity against adult mites of *T. cinnabarinus* with LC_{50} values of 0.0156 and 0.0113 mg l^{-1}, respectively, nearly as potent as their parent compounds.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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