MICROBIAL CONVERSION OF MILBEMYCINS: MICROBIAL CONVERSION OF MILBEMYCINS A_4 AND A_3 BY *Streptomyces libani*

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Milbemycins are a family of sixteen-membered macrolides produced by *Streptomyces hygroscopicus* subsp. *aureolacrimosus*, and they exhibit broad-spectrum insecticidal and acaricidal activity.[1~3]

In the course of studying microbial conversion of milbemycins, in order to prepare some new intermediates for further derivatization and for subsequent use as metabolite reference standards in animal metabolism studies, we have obtained various conversion products: 13β-hydroxymilbemycins, 30-hydroxymilbemycins, 29-hydroxymilbemycins, 28-hydroxymilbemycins, 13β,30-dihydroxymilbemycins, and others.[4~8] On the other hand, RAMOS TOMBO *et al.* have reported 13β-hydroxylation and 14,15-epoxidation of milbemycins by *Streptomyces violascens* ATCC 31560.[9] During our screening of microbial conversion of milbemycin A_4 (**1a**), we observed that an actinomycete, strain SANK 65087, newly isolated from soil, converted milbemycin A_4 (**1a**) to a variety of conversion products.

The present paper deals with purification and identification of converted products from milbemycins A_4 (**1a**) and A_3 (**2a**) using this microorganism.

Strain SANK 65087 was isolated from a soil sample at Adelaide in Australia. On the descriptive media of SHIRLING and GOTTLIEB,[10] and WAKSMAN,[11] after 14 days at 28°C, the aerial mycelium was abundant and formed a grayish white to dark yellowish brown mass. The color of the vegetative mycelium was grayish white to pale yellowish brown. A melanoid pigment was not produced.

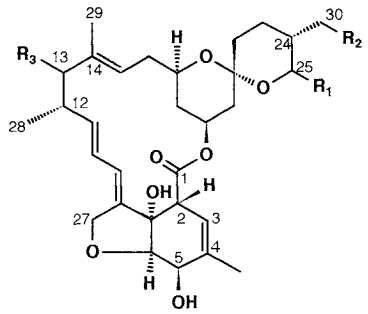
The substrate hyphae were irregularly branched. Mature spore chains were moderately short with 3 to 10 or often more than 10 spores per chain. The spore chains were categorized in Section Spira. The spore surface was smooth. Cell walls and whole cells were analyzed by the procedure of BECKER *et al.*,[12] and LECHEVALIER.[13] The cell wall diaminopimelic acid isomers showed the presence of LL-diaminopimeric acid. The whole cell sugar pattern was not characteristic. The strain was considered to be a cell wall type I.

From these taxonomic studies, the strain was identified as *Streptomyces libani* subsp. *libani*.[14]

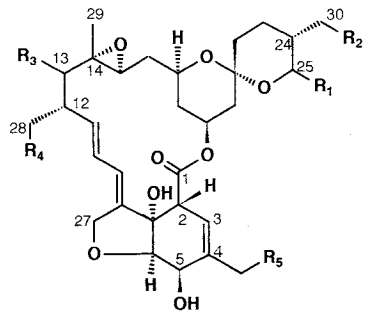
Converted milbemycins in the culture broth were detected by TLC (Merck Art. 5715: EtOAc) and HPLC (column: Waters, Nova pak C_{18} 8 mm × 10 cm; solvent: system 1, acetonitrile - water (75 : 25), with a flow rate of 1.5 ml/minute; system 2, acetonitrile - water (55 : 45), with a flow rate of 1.0 ml/minute; detector: UV 243 nm).

S. libani subsp. *libani* SANK 65087 was cultured in two 500-ml Erlenmeyer flasks containing 100 ml of MY medium[4] at 28°C on a rotary shaker (200 ~ 220 rpm). After two days of cultivation, milbemycin A_4 (**1a**) (5% [w/v] in 1,4-dioxane) was added to a final concentration of 250 μg/ml and cultivation was continued for 7 additional days. Then the culture broth was extracted with three 100-ml portions of EtOAc. The EtOAc extract was dried over anhydrous sodium sulfate and evaporated. The extract was injected into preparative HPLC (column: Sensyu-kagaku ODS-H-5251 20 mm × 250 mm; solvent: acetonitrile - water (75 : 25), with a flow rate of 10 ml/minute; detector: UV 243 nm), to give four new conversion products, which were characterized as 13β-hydroxy-14,15-epoxymilbemycin A_4 (**1b**, 0.6 mg, 1.1%), 30-hydroxy-14,15-epoxymilbemycin A_4 (**1c**, 6.1 mg, 11.5%), 28-hydroxy-14,15-epoxymilbemycin A_4 (**1d**, 1.6 mg, 3.0%), and 26-hydroxy-14,15-epoxymilbemycin A_4 (**1e**, 1.9 mg, 3.6%) from their physico-chemical properties, and to give three other compounds, 13β-hydroxymilbemycin A_4[5] (**1f**, 3.8 mg, 7.4%), 13β,30-dihydroxymilbemycin A_4[5] (**1g**, 3.2 mg, 5.9%), and 14,15-epoxymilbemycin A_4[9,15] (**1h**, 2.6 mg, 5.1%), which were identified by comparing their ^{1}H NMR and mass spectra with those of the authentic compounds.

Milbemycin A_3 (**2a**) (600 mg) was also converted by the strain. The culture broth was extracted and evaporated in a similar method as for milbemycin A_4. EtOAc extract was separated by a reversed-phase silica gel column (Fuji-gel Hanbai Silica gel ODS-Q3, water - methanol) and a subsequent preparative TLC (Merck Art. 5715: EtOAc), to



	R_1	R_2	R_3
1a	CH_2CH_3	H	H
2a	CH_3	H	H
1f	CH_2CH_3	H	OH
2f	CH_3	H	OH
1g	CH_2CH_3	OH	OH
2g	CH_3	OH	OH



	R_1	R_2	R_3	R_4	R_5
1h	CH_2CH_3	H	H	H	H
2h	CH_3	H	H	H	H
1b	CH_2CH_3	H	OH	H	H
2b	CH_3	H	OH	H	H
1c	CH_2CH_3	OH	H	H	H
2c	CH_3	OH	H	H	H
1d	CH_2CH_3	H	H	OH	H
2d	CH_3	H	H	OH	H
1e	CH_2CH_3	H	H	H	OH
2e	CH_3	H	H	H	OH
2i	CH_3	H	H	OH	OH

give eight converted products: 13β-hydroxy-14,15-epoxymilbemycin A_3 (**2b**, 3.8 mg, 1.2%), 30-hydroxy-14,15-epoxymilbemycin A_3 (**2c**, 23.2 mg, 7.3%), 28-hydroxy-14,15-epoxymilbemycin A_3 (**2d**, 37.1 mg, 11.7%), 26-hydroxy-14,15-epoxymilbemycin A_3 (**2e**, 30.2 mg, 9.5%), 26,28-dihydroxy-14,15-epoxymilbemycin A_3 (**2i**, 5.5 mg, 1.7%), 13β-hydroxymilbemycin A_3[15] (**2f**, 5.5 mg, 1.7%), 13β,30-dihydroxymilbemycin A_3 (**2g**, 2.3 mg, 0.7%),

Table 1. TLC Rf values and HPLC retention times of milbemycins and conversion products.

Compound[a]	TLC Rf[b] values	HPLC Rt's[b] (minutes)	
		System 1	System 2
1a	0.59	16.07	—
1b	0.42	2.98	7.69
1c	0.32	2.20	4.22
1d	0.12	2.92	7.00
1e	0.11	3.17	8.88
1f	0.46	3.50	10.86
1g	0.26	2.00	3.38
1h	0.53	5.24	21.30
2a	0.59	11.80	—
2b	0.42	2.63	5.97
2c	0.29	2.03	3.80
2d	0.10	2.57	5.49
2e	0.09	2.77	6.72
2f	0.46	3.02	8.04
2g	0.24	1.97	3.17
2h	0.52	4.17	15.14
2i	0.02	2.08	3.54

[a] **a**, substrates; **b**~**i**, products.
[b] Rf values and retention times relative to **1f**.

and 14,15-epoxymilbemycin A_3 (**2h**, 26.4 mg, 8.5%). The Rf values on TLC and HPLC retention times of these derivatives are listed in Table 1. IR spectra were recorded with a Nicolet 5S × C FT-IR spectrophotometer. NMR spectra were measured at 270 MHz on a JEOL JNM GX 270 spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were measured on a JEOL JMS-D 300 spectrometer. The physico-chemical properties of these compounds are listed below.

13β-Hydroxy-14,15-epoxymilbemycin A_4 (**1b**): IR (KBr) cm^{-1} 3650~3100 (br s), 2963 (s), 2929 (s), 2873 (s), 1719 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.96 (1H, dd, J=14.5, 11.3 Hz, 10-H), 5.80 (1H, dt, J_d=11.3, J_t=2.0 Hz, 9-H), 5.27~5.48 (3H, m, 3-H, 11-H, 19-H), 4.74 (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.73 (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.28~4.32 (1H, m, 5-H), 3.99 (1H, d, J=6.4 Hz, 6-H), 3.69~3.78 (1H, m, 17-H), 3.49 (1H, s, 7-OH), 3.30~3.32 (1H, m, 2-H), 3.07 (1H, td, J_t=8.9, J_d=2.4 Hz, 25-H), 2.84 (1H, d, J=10.1 Hz, 13-H), 2.81 (1H, d, J=9.5 Hz, 15-H), 2.22~2.43 (3H, m, 5-OH, 12-H, 16-H), 2.09~2.14 (1H, m, 20-H), 1.89 (3H, s, 26-H_3), 1.28~1.82 (10H, m, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H, 31-H_2), 1.27 (3H, s, 29-H_3), 1.14 (3H, d, J=6.4 Hz, 28-H_3), 0.95~1.14 (1H, m, 18-H), 0.99 (3H, t, J=7.3 Hz, 32-H_3), 0.83 (3H, d, J=6.4 Hz, 30-H_3); EI-MS m/z 574 (M^+, $C_{32}H_{46}O_9$) (33%), 556, 472, 446, 428, 410, 388, 346, 317, 295,

279, 261, 239, 195, 167, 151 (base peak); HREI-MS calcd for $C_{32}H_{46}O_9$: 574.3142, found: 574.3138.

30-Hydroxy-14,15-epoxymilbemycin A_4 (**1c**): IR (KBr) cm^{-1} 3650~3100 (br s), 2961 (s), 2926 (s), 2872 (s), 1721 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.92 (1H, dd, J=14.1, 11.3 Hz, 10-H), 5.81 (1H, dt, J_d=11.3, J_t=2.2 Hz, 9-H), 5.27~5.48 (3H, m, 3-H, 11-H, 19-H), 4.76 (1H, dd, J=14.8, 2.0 Hz, 27-H), 4.70 (1H, dd, J=14.8, 2.0 Hz, 27-H), 4.30 (1H, t, J=6.3 Hz, 5-H), 3.98 (1H, d, J=6.3 Hz, 6-H), 3.68~3.81 (1H, m, 17-H), 3.63 (1H, dd, J=10.9, 4.4 Hz, 30-H), 3.55 (1H, s, 7-OH), 3.48 (1H, dd, J=10.9, 6.4 Hz, 30-H), 3.27~3.38 (2H, m, 2-H, 25-H), 2.59 (1H, d, J=8.9 Hz, 15-H), 2.31~2.48 (2H, m, 12-H, 16-H), 2.07~2.26 (3H, m, 13-H, 16-H, 20-H), 1.89 (3H, s, 26-H_3), 1.24 (3H, s, 29-H_3), 1.20~1.87 (10H, m, 13-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H, 31-H_2), 0.95~1.17 (7H, m, 18-H, 28-H_3, 32-H_3); EI-MS m/z 574 (M^+, $C_{32}H_{46}O_9$) (7%), 538, 476, 446, 410, 370, 330, 295, 264, 251, 239, 211, 193, 183, 165, 150, 95 (base peak); HREI-MS calcd for $C_{32}H_{46}O_9$: 574.3142, found: 574.3156.

28-Hydroxy-14,15-epoxymilbemycin A_4 (**1d**): IR (KBr) cm^{-1} 3650~3100 (br s), 2960 (s), 2928 (s), 2873 (s), 1720 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 6.02 (1H, dd, J=14.7, 11.2 Hz, 10-H), 5.87 (1H, dt, J_d=11.2, J_t=2.4 Hz, 9-H), 5.33~5.45 (3H, m, 3-H, 11-H, 19-H), 4.75 (1H, dd, J=14.7, 2.4 Hz, 27-H), 4.73 (1H, dd, J=14.7, 2.4 Hz, 27-H), 4.30 (1H, d, J=6.4 Hz, 5-H), 3.99 (1H, d, J=6.4 Hz, 6-H), 3.72~3.80 (1H, m, 17-H), 3.50~3.65 (1H, br s, 7-OH), 3.52 (1H, dd, J=10.8, 5.9 Hz, 28-H), 3.29~3.37 (2H, m, 2-H, 28-H), 3.07 (1H, td, J_t=9.3, J_d=2.4 Hz, 25-H), 2.62 (1H, d, 8.8 Hz, 15-H), 2.38~2.55 (1H, m, 12-H), 2.07~2.26 (3H, m, 13-H, 16-H, 20-H), 1.89 (3H, s, 26-H_3), 1.80~1.89 (1H, m, 18-H), 1.01~1.70 (10H, m, 13-H, 16-H, 20-H, 22-H_2, 23-H_2, 24-H, 31-H_2), 1.25 (3H, s, 29-H_3), 1.00 (3H, t, J=7.3 Hz, 32-H_3), 0.95~1.15 (1H, m, 18-H), 0.83 (3H, d, J=6.3 Hz, 30-H_3); EI-MS m/z 574 (M^+, $C_{32}H_{46}O_9$) (4%), 556 (M^+-H_2O, $C_{32}H_{44}O_8$), 538, 520, 456, 446, 428, 387, 346, 317, 281, 254, 239, 207, 195 (base peak), 177, 167; HREI-MS calcd for $C_{32}H_{44}O_8$: 556.3036, found: 556.3042.

26-Hydroxy-14,15-epoxymilbemycin A_4 (**1e**): IR (KBr) cm^{-1} 3650~3100 (br s), 2960 (s), 2927 (s), 2871 (s), 1722 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.75~5.96 (3H, m, 3-H, 9-H, 10-H), 5.49 (1H, dd, J=13.7, 9.8 Hz, 11-H), 5.31~5.44 (1H, m, 19-H), 4.68~4.80 (2H, m, 27-H_2), 4.59 (1H, d, J=6.5 Hz, 5-H), 4.30 (1H, d, J=14.2 Hz, 26-H), 4.27 (1H, d, J=14.2 Hz, 26-H), 4.01 (1H, d, J=6.4 Hz, 6-H), 3.70~3.82 (1H, m, 17-H), 3.45~3.65 (1H, br, 7-OH), 3.34~3.37 (1H, m, 2-H), 3.06 (1H, td, J_t=9.3, J_d=2.4 Hz, 25-H), 2.40~2.75 (1H, br s, 5-OH), 2.60 (1H, d, J=9.3 Hz, 15-H), 2.30~2.50 (1H, m, 12-H), 2.05~2.25 (3H, m, 13-H, 16-H, 20-H), 1.82~1.89 (1H, m, 18-H), 1.01~1.65 (10H, m, 13-H, 16-H, 20-H, 22-H_2, 23-H_2, 24-H, 31-H_2), 1.24 (3H, s, 29-H_3), 0.97~1.13 (7H, m, 18-H, 28-H_3, 32-H_3), 0.83 (3H, d, J=6.4 Hz, 30-H_3); EI-MS m/z 574 (M^+, $C_{32}H_{46}O_9$) (0.4%), 556 (M^+-H_2O, $C_{32}H_{44}O_8$), 538, 430, 371, 276, 261, 211, 195 (base peak), 183, 167, 151; HREI-MS calcd for $C_{32}H_{44}O_8$: 556.3036, found: 556.3052.

13β-Hydroxy-14,15-epoxymilbemycin A_3 (**2b**): IR (KBr) cm^{-1} 3650~3100 (br s), 2964 (s), 2927 (s), 2872 (s), 1730 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.97 (1H, dd, J=14.5, 11.3 Hz, 10-H), 5.80 (1H, dt, J_d=11.3, J_t=2.4 Hz, 9-H), 5.29~5.45 (3H, m, 3-H, 11-H, 19-H), 4.74 (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.73 (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.28~4.32 (1H, m, 5-H), 3.99 (1H, d, J=6.5 Hz, 6-H), 3.65~3.78 (1H, m, 17-H), 3.50 (1H, s, 7-OH), 3.20~3.32 (2H, m, 2-H, 25-H), 2.80 (2H, d, J=10.5 Hz, 13-H, 15-H), 2.09~2.43 (4H, m, 5-OH, 12-H, 16-H, 20-H), 1.89 (3H, s, 26-H_3), 1.75~1.83 (1H, m, 18-H), 1.28~1.65 (7H, m, 16-H, 20-H, 22-H_2, 23-H_2, 24-H), 1.27 (3H, s, 29-H_3), 1.12~1.17 (6H, m, 28-H_3, 31-H_3), 0.95~1.14 (1H, m, 18-H), 0.84 (3H, d, J=6.9 Hz, 30-H_3); EI-MS m/z 560 (M^+, $C_{31}H_{44}O_9$) (7%), 524, 432, 414, 368, 279, 225, 199, 181, 167, 149 (base peak); HREI-MS calcd for $C_{31}H_{44}O_9$: 560.2985, found: 560.2983.

30-Hydroxy-14,15-epoxymilbemycin A_3 (**2c**): IR (KBr) cm^{-1} 3650~3100 (br s), 2964 (s), 2926 (s), 2870 (s), 1733 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.91 (1H, dd, J=14.1, 11.3 Hz, 10-H), 5.82 (1H, dt, J_d=11.3, J_t=2.4 Hz, 9-H), 5.30~5.51 (3H, m, 3-H, 11-H, 19-H), 4.73 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.71 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.29 (1H, br s, 5-H), 3.98 (1H, d, J=6.5 Hz, 6-H), 3.46~3.77 (5H, m, 7-OH, 17-H, 25-H, 30-H_2), 3.28~3.31 (1H, m, 2-H), 2.62 (1H, d, J=8.9 Hz, 15-H), 2.30~2.48 (2H, m, 5-OH, 12-H), 2.05~2.24 (3H, m, 13-H, 16-H, 20-H), 1.89 (3H, s, 26-H_3), 1.82~1.89 (1H, m, 18-H), 1.21~1.26 (6H, m, 29-H_3, 31-H_3), 1.02 (3H, d, J=6.9 Hz, 28-H_3), 1.00~1.90 (9H, m, 13-H, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H); EI-MS m/z 560 (M^+, $C_{31}H_{44}O_9$) (13%), 524, 480, 432, 414, 396, 370, 330, 283, 250, 221, 211, 197 (base peak), 181, 169,151; HREI-MS calcd for $C_{31}H_{44}O_9$: 560.2985, found: 560.2960.

28-Hydroxy-14,15-epoxymilbemycin A_3 (**2d**): IR

(KBr) cm^{-1} 3650~3100 (br s), 2964 (s), 2929 (s), 2874 (s), 1733 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 6.05 (1H, dd, J=14.5 Hz, 11.3 Hz, 10-H), 5.86 (1H, dt, J_d=11.3, J_t=2.4 Hz, 9-H), 5.30~5.44 (3H, m, 3-H, 11-H, 19-H), 4.75 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.72 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.27~4.30 (1H, m, 5-H), 3.96 (1H, d, J=6.5 Hz, 6-H), 3.70~3.78 (2H, m, 7-OH, 17-H), 3.37 (1H, dd, J=10.5, 5.3 Hz, 28-H), 3.20~3.37 (3H, m, 2-H, 25-H, 28-H), 2.65 (1H, d, J=8.9 Hz, 15-H), 2.30~2.52 (2H, m, 5-OH, 12-H), 2.08~2.26 (3H, m, 13-H, 16-H, 20-H), 1.88 (3H, s, 26-H_3), 1.82~1.88 (1H, m, 18-H), 1.26 (3H, s, 29-H_3), 1.16 (3H, d, J=6.1 Hz, 31-H_3), 1.01~1.68 (9H, m, 13-H, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H), 0.84 (3H, d, J=6.4 Hz, 30-H_3); EI-MS m/z 560 (M^+, $C_{31}H_{44}O_9$) (4%), 542, 506, 432, 414, 396, 317, 281, 225, 207, 181, 163, 153, 69 (base peak); HREI-MS calcd for $C_{31}H_{44}O_9$: 560.2985, found: 560.2966.

26-Hydroxy-14,15-epoxymilbemycin A_3 (**2e**): IR (KBr) cm^{-1} 3650~3100 (br s), 2964 (s), 2926 (s), 2870 (s), 1733 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.91 (1H, dd, J=14.1, 11.3 Hz, 10-H), 5.83 (1H, dt, J_d=11.3, J_t=2.0 Hz, 9-H), 5.75 (1H, d, J=0.8 Hz, 3-H), 5.49 (1H, dd, J=14.1, 10.1 Hz, 11-H), 5.28~5.40 (1H, m, 19-H), 4.74, (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.72 (1H, dd, J=14.5, 2.0 Hz, 27-H), 4.58~4.60 (1H, m, 5-H), 4.23~4.34 (2H, m, 26-H_2), 4.00 (1H, d, J=6.1 Hz, 6-H), 3.69~3.78 (2H, m, 7-OH, 17-H), 3.33~3.35 (1H, m, 2-H), 3.20~3.31 (1H, m, 25-H), 2.75~2.88 (1H, br, 5-OH), 2.63 (1H, d, J=9.3 Hz, 15-H), 2.31~2.50 (1H, m, 12-H), 2.09~2.25 (3H, m, 13-H, 16-H, 20-H), 1.83~1.89 (1H, m, 18-H), 1.24 (3H, s, 29-H_3), 1.16 (3H, d, J=6.5 Hz, 31-H_3), 1.02 (3H, d, J=6.9 Hz, 28-H_3), 1.00~1.67 (9H, m, 13-H, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H), 0.84 (3H, d, J=6.4 Hz, 30-H_3); EI-MS m/z 560 (M^+, $C_{31}H_{44}O_9$) (3%), 524, 480, 432, 414, 396, 370, 330, 283, 250, 221, 211, 197, 181 (base peak), 169, 151; HREI-MS calcd for $C_{31}H_{44}O_9$: 560.2985, found: 560.2960.

13β,30-Dihydroxymilbemycin A_3 (**2g**): IR (KBr) cm^{-1} 3650~3100 (br s), 2954 (s), 2925 (s), 2869 (s), 1730 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.74~5.85 (2H, m, 9-H, 10-H), 5.23~5.40 (4H, m, 3-H, 11-H, 15-H, 19-H), 4.64~4.74 (2H, m, 27-H_2), 4.26~4.33 (1H, br s, 5-H), 3.99 (1H, s, 7-OH), 3.96 (1H, d, J=6.5 Hz, 6-H), 3.72 (1H, d, J=9.7 Hz, 13-H), 3.48~3.67 (4H, m, 17-H, 25-H, 30-H_2), 3.25~3.29 (1H, m, 2-H), 2.26~2.42 (4H, m, 5-OH, 12-H, 16-H_2), 1.99~2.05 (1H, m, 20-H), 1.88 (3H, s, 26-H_3), 1.58 (3H, s, 29-H_3), 1.25~1.78 (7H, m, 18-H, 20-H, 22-H_2, 23-H_2, 24-H), 1.22 (3H, d, J=6.4 Hz, 31-H_3), 1.13 (3H, d, J=6.9 Hz, 28-H_3), 0.80~1.00 (1H, m, 18-H); EI-MS m/z 560 (M^+, $C_{31}H_{44}O_9$) (0.9%), 542, 414, 281 (base peak), 263, 237, 197, 169; HREI-MS calcd for $C_{31}H_{44}O_9$: 560.2985, found: 560.2989.

14,15-Epoxymilbemycin A_3 (**2h**): IR (KBr) cm^{-1} 3650~3100 (br s), 2965 (s), 2927 (s), 2874 (s), 1733 (s), 1719 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 5.90 (1H, dd, J=14.1, 11.3 Hz, 10-H), 5.82 (1H, dt, J_d=11.3, J_t=2.4 Hz, 9-H), 5.31~5.51 (3H, m, 3-H, 11-H, 19-H), 4.73 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.71 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.27~4.33 (1H, m, 5-H), 3.98 (1H, d, J=6.0 Hz, 6-H), 3.68~3.77 (1H, m, 17-H), 3.56 (1H, s, 7-OH), 3.20~3.31 (2H, m, 2-H, 25-H), 2.63 (1H, d, J=8.9 Hz, 15-H), 2.35~2.47 (2H, m, 5-OH, 12-H), 2.06~2.24 (3H, m, 13-H, 16-H, 20-H), 1.89 (3H, s, 26-H_3), 1.82~1.89 (1H, m, 18-H), 1.24 (3H, s, 29-H_3), 1.16 (3H, d, J=6.0 Hz, 31-H_3), 1.02 (3H, d, J=6.8 Hz, 28-H_3), 1.00~1.89 (9H, m, 13-H, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H), 0.84 (3H, d, J=6.4 Hz, 30-H_3); EI-MS m/z 544 (M^+, $C_{31}H_{44}O_8$) (20%), 508, 416, 398, 380, 354, 330, 267, 247, 181 (base peak), 164, 153; HREI-MS calcd for $C_{31}H_{44}O_8$: 544.3036, found: 544.3035.

26,28-Dihydroxy-14,15-epoxymilbemycin A_3 (**2i**): IR (KBr) cm^{-1} 3650~3100 (br s), 2962 (s), 2929 (s), 2874 (s), 1725 (s); ^1H NMR (270 MHz, $CDCl_3$) δ 6.05 (1H, dd, J=14.5, 11.3 Hz, 10-H), 5.88 (1H, dt, J_d=11.3, J_t=2.4 Hz, 9-H), 5.78 (1H, s, 3-H), 5.28~5.48 (2H, m, 11-OH, 19-H), 4.76 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.73 (1H, dd, J=14.5, 2.4 Hz, 27-H), 4.58 (1H, d, J=6.1 Hz, 5-H), 4.28 (2H, s, 26-H_2), 3.97 (1H, d, J=6.1 Hz, 6-H), 3.85~4.00 (1H, br, 7-OH), 3.70~3.79 (1H, m, 17-H), 3.54 (1H, dd, J=10.5, 5.2 Hz, 28-H), 3.21~3.38 (3H, m, 2-H, 25-H, 28-H), 2.60~2.95 (1H, br, 5-OH), 2.66 (1H, d, J=9.3 Hz, 15-H), 2.40~2.55 (1H, m, 12-H), 2.10~2.26 (3H, m, 13-H, 16-H, 20-H), 1.83~1.90 (1H, m, 18-H), 1.26 (3H, s, 29-H_3), 1.16 (3H, d, J=6.0 Hz, 31-H_3), 1.02~1.70 (9H, m, 13-H, 16-H, 18-H, 20-H, 22-H_2, 23-H_2, 24-H), 0.84 (3H, d, J=6.5 Hz, 30-H_3); EI-MS m/z 576 (M^+, $C_{31}H_{44}O_{10}$) (2%), 558, 540, 496, 432, 396, 368, 333, 271, 253, 225, 181 (base peak), 163, 153; HREI-MS calcd for $C_{31}H_{44}O_{10}$: 576.2935, found: 576.2914.

All of the identified compounds here were tested in acaricidal screening using two-spotted spider mite (*Tetraxychus urticae*). They exhibited only moderate activity. Consequently, the activity of hydroxylated and epoxidated compounds shown here were inferior to parent milbemycin A_4 (**1a**)

and A_3 (**2a**).

The present work shows that *S. libani* converted milbemycins into monohydroxy- and dihydroxy-14,15-epoxy derivatives, in addition to the 13β-hydroxy and 14,15-epoxy derivatives which have been reported by RAMOS TOMBO *et al.*[9] in the microbial conversion with *S. violascens* ATCC 31560. We have also discovered ten or so other actinomycetes strains newly isolated from soil which have this type of conversion activity.

Although the efficiency of the conversion by these microorganisms was not enough to provide oxygenated milbemycins as intermediates for chemical synthesis, it is of interest that different strains of actinomycetes showed a common type of conversion activity.

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