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Milbemycin α_{17} and Related Compounds Synthesized from Milbemycin A_4: Synthetic Procedure and Acaricidal Activities

TAKAHIRO TSUKIYAMA,* AYAKO KINOSHITA, REIJI ICHINOSE† and KAZUO SATO††

Agroscience Research Laboratories, Sankyo Agro Co., Ltd.
894 Yasu, Yasu-cho, Yasu-gun, Shiga 520-2342, Japan
† Crop Protection Department, Sankyo Agro Co., Ltd.
Kasuga Bldg., 4-23-14, Hongo, Bunkyo-ku,
Tokyo 113-0033, Japan
†† Intellectual Property Department, Sankyo Co., Ltd.
2-58, Hiromachi 1-Chome, Shinagawa-ku,
Tokyo 140-8710, Japan

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Milbemycin α_{17}, a 14-demethyl congener of milbemycin A_4, has been reported as a natural product. In this paper, we report the successful development of a chemical derivation method to synthesize milbemycin α_{17} from milbemycin A_4, as well as our use of a similar method to prepare 24-demethylmilbemycin A_4 from the same precursor. The acaricidal activities of these compounds were assessed against the organophosphorus-sensitive two-spotted spider mites (*Tetranychus urticae*) on the primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying.

Milbemycins[1~7] are a family of sixteen-membered ring macrolides that have been isolated from *Streptomyces hygroscopicus*. They exhibit notable activities as acaricides, insecticides, and anthelmintics. Among them, milbemectin[8] [a mixture of milbemycin A_3 (**1**) and A_4 (**2**) (Figure 1)] was developed as an agricultural acaricide. Since the discovery of milbemycins, enormous efforts have been made to search for homologues that possess the same sixteen-membered macrolides moieties from nature. These efforts have been fruitful and have led to the isolation and documentation of numerous congeners, including Merck's avermectins[9] and Cyanamid's LL-F28249 series (nemadectins).[10] Milbemycin α_{17} (**3**) (Figure 1), another of the congeners, has been reported as a natural product.[11] The structure of milbemycin α_{17} (**3**) is characterized by a lack of the methyl group at its C-14 position. In investigating the structure-activity relationship of the milbemycins, the role of the methyl group at the C-14 position is a compelling topic for study. The biosynthesis pathway of milbemycin α_{17} (**3**) is another attractive topic for study, but the low bio-productivity[11] of milbemycin α_{17} (**3**) has so far set limits on attempts to conduct this type of research. Based on the foregoing, our group saw the need for a chemical derivation method to synthesize milbemycin α_{17} (**3**) from milbemycin A_4 (**2**). We developed such a method by applying the decarbonylation reaction[12] with chlorotris(triphenylphosphine)rhodium(I) [$RhCl(PPh_3)_3$, Wilkinson's catalyst] to milbemycin derivatives.

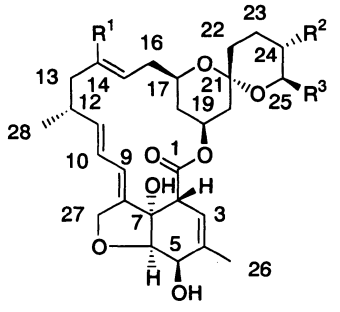
Meanwhile, several other groups disclosed derivations around the C-24 methyl group[13~16] of the milbemycin framework. Most of their studies focused on the derivations of the substituent at the C-25 position, however, and none studied the relationship between the presence of the C-24 methyl group and the biological activities of the milbemycins. Several methods[13~16] for the synthesis of the 24-demethylmilbemycin framework were disclosed in the same studies, but all of these methods employed tedious strategies, such as the cleavage of the spiroketal moiety using the oxygen-containing functional groups located at the C-22 or C-23 positions, elongation of the carbon chains,

* Corresponding author: tukiya@sankyo-agro.co.jp

and reconstruction of the spiroketal moiety. Moreover, none of these methods could be applied to milbemycin A_4 (**2**), as there were no oxygen-containing functional groups at the C-22 or C-23 positions. Accordingly, we examined the synthesis of 24-demethylmilbemycin A_4 (**4**) (Figure 1) from milbemycin A_4 (**2**) under a condition similar to that used to prepare milbemycin α_{17} (**3**).

In addition, we assessed the acaricidal activities of these compounds against the two-spotted spider mites (*Tetranychus urticae*) on the primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying in order to clarify the roles of the C-14 and C-24 methyl groups. We report the results in this paper.

Fig. 1. Structures and numberings of milbemycins.



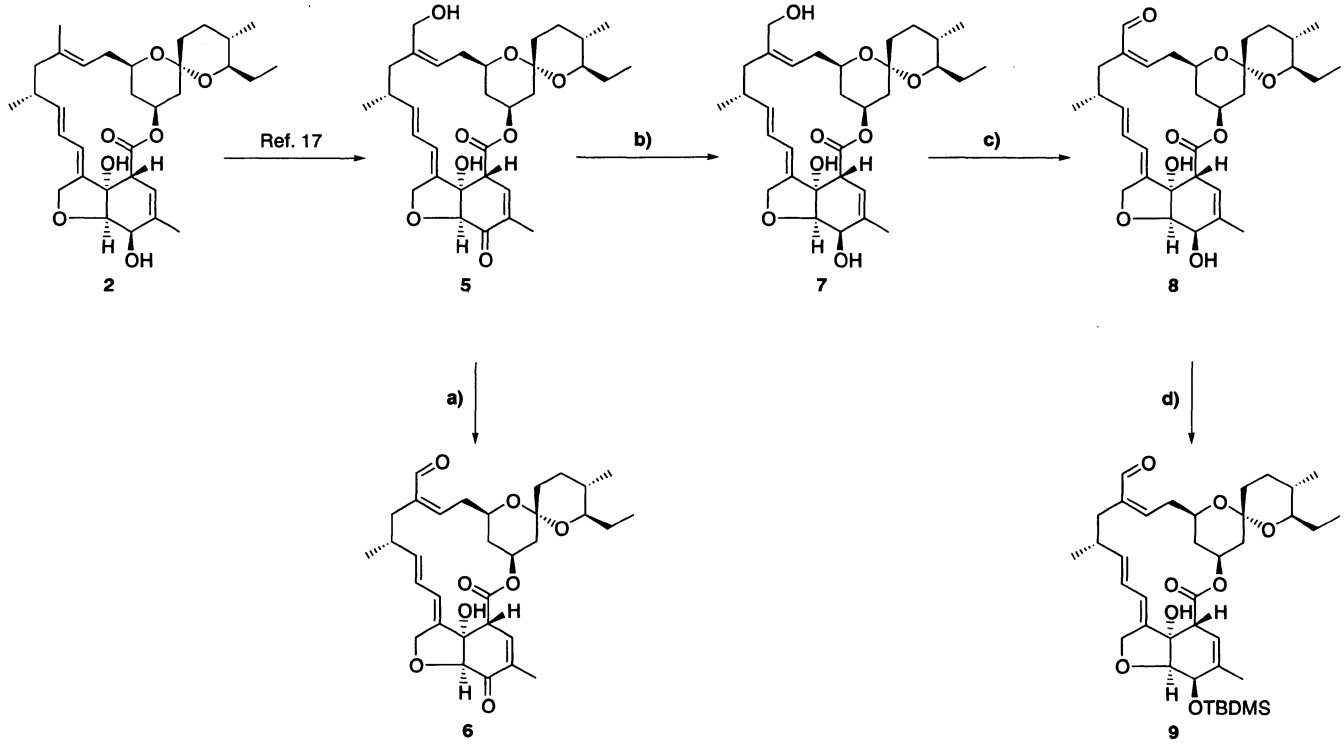
Milbemycin A_3 (**1**) $R^1=C^{(29)}H_3$, $R^2=C^{(30)}H_3$, $R^3=C^{(31)}H_3$
Milbemycin A_4 (**2**) $R^1=C^{(29)}H_3$, $R^2=C^{(30)}H_3$, $R^3=C^{(31)}H_2C^{(32)}H_3$
Milbemycin α_{17} (**3**) $R^1=H$, $R^2=C^{(29)}H_3$, $R^3=C^{(30)}H_2C^{(31)}H_3$
24-Demethylmilbemycin A_4 (**4**) $R^1=C^{(29)}H_3$, $R^2=H$, $R^3=C^{(30)}H_2C^{(31)}H_3$

Results and Discussion

Chemistry

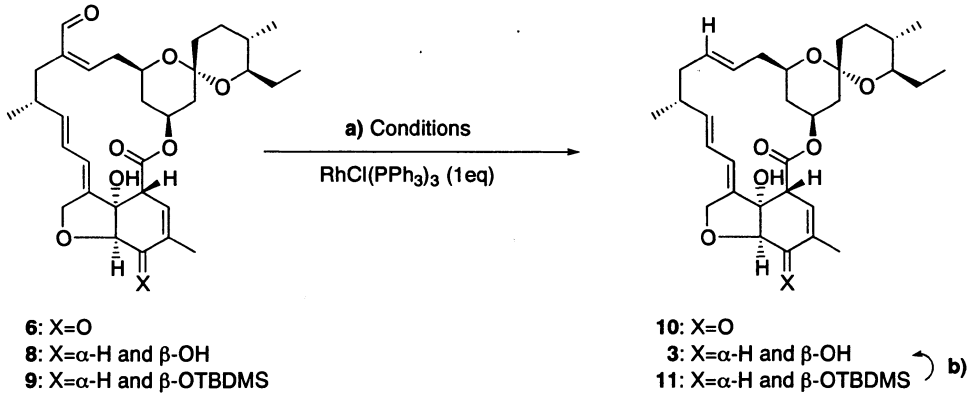
Our first step was to examine the preparations of the precursors (**6**, **8**, **9**) for decarbonylation reactions from milbemycin A_4 (**2**) (Scheme 1). The 5-deoxy-29-hydroxy-5-oxomilbemycin A_4 (**5**) was derived from milbemycin A_4 (**2**) by using the selenium dioxide oxidation reported by TSUKAMOTO *et al.*[17] Oxidation of the hydroxy group at the C-29 position produced 5-deoxy-14-formyl-5-oxomilbemycin A_4 (**6**)[17] with pyridinium chlorochromate[18] (PCC) in moderate yield. Meanwhile, the ketone moiety at the C-5 position of **5** was selectively reduced to the corresponding

Scheme 1. Synthesis of precursors for decarbonylation reactions.



Reagents: **a)** PCC, CH_2Cl_2; 69.5%; **b)** $NaBH_4$, MeOH; 84.3%; **c)** TEMPO, NCS, TBACl, CH_2Cl_2, buffer; 86.8%; **d)** TBDMSCl, imidazole, DMF; 85.1%.

Scheme 2. Examinations of decarbonylation reactions and synthesis of milbemycin α_{17} (**3**).



Reagents: a) see Table 1.; **b)** p-TsOH·H_2O, MeOH; 92.9%.

Table 1. Examinations of decarbonylation reactions.

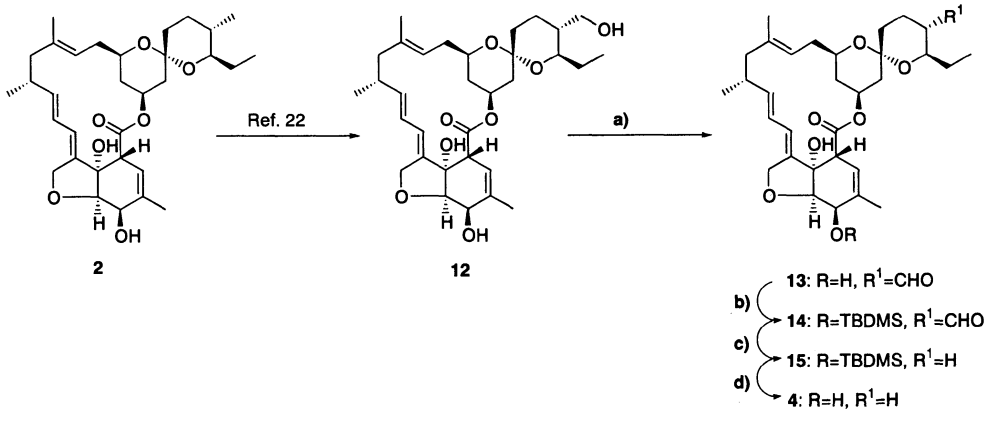
Entry	Substrate	Conditions	Products (yield %)
1	6	benzene, reflux, 5hr.	10 (5.2%) + 6 (27.7%)[a]
2	6	benzene, reflux, overnight	messy[b]
3	6	toluene, reflux, 1hr.	10 (trace)
4	6	acetonitrile, reflux, overnight	messy[b]
5	6	benzonitrile, 165°C, 15min.	messy[b]
6	8	toluene, reflux, 1hr.	3 (5.6%) + 8 (7.4%)[a]
7	8	benzonitrile, 165°C, 10min.	3 (6.7%)
8	9	benzonitrile, 165°C, 10min.	11 (81.6%)

a) The starting material was recovered.
b) The starting material and product were degraded into complex mixtures.

β-hydroxy group with sodium borohydride ($NaBH_4$).[3] The primary hydroxy group of 29-hydroxymilbemycin A_4 (**7**) was selectively oxidized to give 14-formylmilbemycin A_4 (**8**) in the presence of *N*-chlorosuccinimide (NCS), 2,2,6,6,-tetramethyl-1-piperidinyloxy free radical (TEMPO) and tetrabutylammonium chloride (TBACl) in good yield.[19] Subsequent protection of the C-5 hydroxy group with a *t*-butyldimethylsilyl (TBDMS) moiety[20] afforded 5-*O*-TBDMS-14-formylmilbemycin A_4 (**9**) in good yield.

Next, we attempted decarbonylation reactions[12] with $RhCl(PPh_3)_3$ (Wilkinson's catalyst) on synthesized precursors (**6**, **8**, **9**) (Scheme 2). The results are summarized in Table 1. Refluxing 5-deoxy-14-formyl-5-oxomilbemycin A_4 (**6**) in benzene with $RhCl(PPh_3)_3$ gave a small amount of decarbonylated product (**10**) together with the remaining starting material (**6**) (entry 1). No improvements in yield were obtained by prolonging of the reaction time (entry 2) or raising the reaction temperature (refluxing in toluene; entry 3). On the contrary, the residual substrate and the products were degraded into complex mixtures. The use of the solvents containing cyano groups[12] (entries 4, 5) also proved unsuccessful. As it seemed that both the substrate (**6**) and product (**10**) were unstable under these reaction conditions, we tried to use 14-formylmilbemycin A_4 (**8**) as the substrate for this reaction instead. Initially this strategy also produced poor results (entries 6, 7), perhaps due to the instabilities of **8** and milbemycin α_{17} (**3**) under these conditions, but we later discovered that protection of the

Scheme 3. Synthesis of 24-demethylmilbemycin A_4 (**4**).



Reagents: a) TEMPO, NCS, TBACl, CH_2Cl_2, buffer, 53.3%; **b)** TBDMSCl, imidazole, DMF, 94.6%; **c)** $RhCl(PPh_3)_3$, benzonitrile, 68.1%; **d)** p-TsOH·H_2O, MeOH, 95.3%.

C-5 hydroxy group with a TBDMS moiety drastically improved the yield (entry 8). Though unable to clearly confirm the degradation pathways of **6**, **8**, **10** and **3** under these reaction conditions, we found that the protection of C-5 hydroxy groups reliably stabilized **9** and **11**, which ultimately improved the yield of **11**. The TBDMS group of **11** was removed with *p*-toluenesulfonic acid monohydrate[21] (*p*-TsOH·H_2O) to afford milbemycin α_{17} (**3**) in good yield (Scheme 2). All spectral data of synthesized milbemycin α_{17} (**3**) were in accord with those of the reference material.[11]

The 24-demethylmilbemycin A_4 (**4**) was prepared from milbemycin A_4 (**2**) using a similar procedure (Scheme 3). First, 30-hydroxymilbemycin A_4 (**12**), prepared from milbemycin A_4 (**2**) by microbial oxidation,[22] was converted to the corresponding 24-formylmilbemycin A_4 (**13**) with the combined addition of NCS/TEMPO/TBACl[19] in moderate yield. Subsequent protection of its C-5 hydroxy group with the TBDMS moiety[20] afforded 5-*O*-TBDMS-24-formylmilbemycin A_4 (**14**) in good yield. Decarbonylation of **14** with Wilkinson's catalyst[12] followed by deprotection of the C-5 hydroxy group[21] gave 24-demethylmilbemycin A_4 (**4**) in good to moderate yields, respectively.

Acaricidal Activities

The synthesized milbemycin α_{17} (**3**) and 24-demethylmilbemycin A_4 (**4**) were studied to assess their acaricidal activities against the two-spotted spider mites (*Tetranychus urticae*). The results are listed in Table 2.

Table 2. Acaricidal activities of milbemycins against the two-spotted spider mite.

	Mortality(%)	
	10ppm	1ppm
Milbemycin α_{17} (3)	37	12
24-Demethylmilbemycin A_4 (4)	34	11
Milbemycin A_3 (1)	69	3
Milbemycin A_4 (2)	100	32

Milbemycin α_{17} (**3**) and 24-demethylmilbemycin A_4 (**4**) retained acaricidal activities, though the activities of both compounds were inferior to those of their parent molecule milbemycin A_4 (**2**). These results suggest that the presence of methyl groups at the C-14 and C-24 positions of the milbemycin framework were not essential for its high acaricidal activities, but that their absence might have interfered with the fitting of the compounds to the target sites.

Experimental

NMR spectra were measured on a Varian Gemini-200 FT NMR Spectrometer (200 MHz). Chemical shifts (δ) were expressed in parts per million relative to internal tetramethylsilane. Mass spectra were measured on a Fisions

Instruments VG Autospec. IR spectra were measured on a Perkin Elmer 1600 series FT IR.

5-Deoxy-14-formyl-5-oxomilbemycin A_4 (**6**)

To a stirred suspension of PCC (2.04 g, 9.44 mmol) in dichloromethane (CH_2Cl_2, 40 ml), a solution of **5** (1.05 g, 1.89 mmol) in CH_2Cl_2 (60 ml) was added dropwise at ambient temperature under a nitrogen atmosphere. After stirring for 15 minutes, diethyl ether (Et_2O, 100 ml) and magnesium sulfate ($MgSO_4$, 20 g) were added to the reaction mixture and stirred. After stirring for another 10 minutes, the reaction mixture was filtered through a Celite® pad, washed with Et_2O, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [*n*-hexane (Hex) - ethyl acetate (EtOAc) gradient] to give 0.73 g (69.5%) of **6** as a colorless amorphous solid.

29-Hydroxymilbemycin A_4 (**7**)

To a stirred solution of **5** (200 mg, 0.36 mmol) in methanol (MeOH, 4 ml), $NaBH_4$, (20.4 mg, 0.54 mmol) was added while cooling in an ice bath under a nitrogen atmosphere. After stirring for 10 minutes, the reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by preparative TLC (PTLC, Hex - EtOAc = 1 - 2) to give 169.2 mg (84.3%) of **7** as a pale yellow amorphous solid.

7: IR ν_{max} (film) cm^{-1}: 3445, 2965, 2930, 2870, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.62~5.87 (2H, m, H-9, H-10), 5.31~5.52 (3H, m, H-3, H-11, H-19), 5.12 (1H, t, J=8.1 Hz, H-15), 4.67 (2H, m, H_2-27), 4.22~4.38 (2H, m, H-5, H-29), 4.11 (1H, s, 7-OH), 3.96 (1H, d, J=5.9 Hz, H-6), 3.94 (1H, m, H-29), 3.59 (1H, m, H-17), 3.25 (1H, m, H-2), 3.06 (1H, m, H-25), 2.48~2.65 (3H, m, H-12, H_2-16), 2.23~2.40 (3H, m, H-13, 5-OH, 29-OH), 1.87 (3H, br, H_3-26), 1.03 (3H, d, J=6.2 Hz, H_3-28), 0.99 (3H, t, J=7.3 Hz, H_3-32), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.76~2.05 (12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 558 (M^+), 430, 412, 330; HREI-MS (*m/z*): [M^+]: Calcd for $C_{32}H_{46}O_8$, 558.3193; found, 558.3193.

14-Formylmilbemycin A_4 (**8**)

To a vigorously stirred solution of **7** (500 mg, 0.90 mmol) in CH_2Cl_2 (5 ml) and 5 ml of an aqueous solution of sodium hydrogen carbonate ($NaHCO_3$, 0.5 M) and potassium carbonate (K_2CO_3, 0.05 M) were added TEMPO (28 mg, 0.18 mmol), TBACl (50 mg, 0.18 mmol) and NCS (239 mg, 1.79 mmol) at ambient temperature. After stirring for 1 hour, the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hex - EtOAc gradient) to give 432.5 mg (86.8%) of **8** as a colorless amorphous solid.

8: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2915, 2865, 1735, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 10.08 (1H, s, CHO), 6.18 (1H, dd, J=12.8 Hz, 3.7 Hz, H-15), 5.60~5.87 (2H, m, H-9, H-10), 5.22~5.55 (3H, m, H-3, H-11, H-19), 4.64 (2H, m, H_2-27), 4.28 (1H, m, H-5), 4.07 (1H, s, 7-OH), 3.95 (1H, d, J=6.2Hz, H-6), 3.75 (1H, m, H-25), 3.28 (1H, m, H-2), 2.93~3.16 (2H, m, H-12, H-17), 2.42~2.81 (3H, m, H-13, H_2-16), 2.37 (1H, d, J=8.1 Hz, 5-OH), 2.05 (1H, m, H-20), 1.87 (3H, br, H_3-26), 1.03 (3H, d, J=6.6 Hz, H_3-28), 1.01 (3H, t, J=7.3 Hz, H_3-32), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.75~1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 556 (M^+), 538, 520, 428, 410, 328; HREI-MS (*m/z*): [M^+]: Calcd for $C_{32}H_{44}O_8$, 556.3036; found, 556.3035.

24-Formylmilbemycin A_4 (**13**)

Using the same procedure described for the preparation of **8**, **13** was prepared from **12** (500 mg, 0.90 mmol) as a colorless amorphous solid in 53.3% yield (265.6 mg, 0.48 mmol).

13: IR ν_{max} (film) cm^{-1}: 3465, 2965, 2915, 2865, 1715; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.63 (1H, d, J=2.9 Hz, CHO), 5.68~5.85 (2H, m, H-9, H-10), 5.28~5.45 (3H, m, H-3, H-11, H-19), 4.97 (1H, m, H-15), 4.68 (2H, m, H_2-27), 4.30 (1H, br, H-5), 4.00 (1H, s, 7-OH), 3.96 (1H, d, J=6.2 Hz, H-6), 3.49~3.74 (2H, m, H-17, H-25), 3.27 (1H, m, H-2), 1.88 (3H, br, H_3-26), 1.54 (3H, br, H_3-29), 1.03 (3H, t, J=7.7 Hz, H_3-32), 0.99 (3H, d, J=7.3 Hz, H_3-28), 0.83~2.55 (17H, m, H-12, H_2-13, H_2-16, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31, 5-OH); EI-MS (*m/z*): 556 (M^+), 538, 520, 428; HREI-MS (*m/z*): [M^+]: Calcd for $C_{32}H_{44}O_8$, 556.3036; found, 556.3035.

5-*O*-TBDMS-14-formylmilbemycin A_4 (**9**)

To a stirred solution of **8** (300 mg, 0.54 mmol) in *N*,*N*-dimethylformamide (DMF, 6 ml) were added *t*-butyldimethylsilylchloride (TBDMSCl, 243 mg, 1.62 mmol) and imidazole (110 mg, 1.62 mmol) at ambient temperature under a nitrogen atmosphere. After stirring for 3 hours, the reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated under

reduced pressure. The residue was purified by silica gel column chromatography (Hex - EtOAc gradient) to give 307.8 mg (85.1%) of **9** as a colorless amorphous solid.

9: IR ν_{max} (film) cm^{-1}: 3455, 2955, 2925, 2855, 1735, 1705, 1675; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 10.08 (1H, s, CHO), 6.16 (1H, m, H-15), 5.60~5.79 (2H, m, H-9, H-10), 5.24~5.45 (3H, m, H-3, H-11, H-19), 4.60 (2H, m, H_2-27), 4.41 (1H, m, H-5), 4.13 (1H, s, 7-OH), 3.80 (1H, d, J=5.5 Hz, H-6), 3.75 (1H, m, H-17), 3.38 (1H, m, H-2), 2.97~3.16 (2H, m, H-16, H-25), 2.42~2.80 (3H, m, H-12, H-13, H-16), 2.05 (1H, m, H-20), 1.79 (3H, br, H_3-26), 1.02 (3H, d, J=7.0 Hz, H_3-28), 0.92 (9H, s, $SiC(CH_3)_3$), 0.83 (3H, d, J=6.2 Hz, H_3-30), 0.13 (3H, s, $SiCH_3$), 0.10 (3H, s, $SiCH_3$), 0.80~1.82 (14H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31, H_3-32); EI-MS (*m/z*): 670 (M^+), 652, 595, 577, 520, 428, 410; HREI-MS (*m/z*): [M^+]: Calcd for $C_{38}H_{58}O_8Si$, 670.3901; found, 670.3902.

5-*O*-TBDMS-24-formylmilbemycin A_4 (**14**)

Using the same procedure described for the preparation of **9**, **14** was prepared from **13** (250 mg, 0.45 mmol) as a colorless amorphous solid in 94.6% yield (285 mg, 0.43 mmol).

14: IR ν_{max} (film) cm^{-1}: 3455, 2955, 2925, 2860, 1720; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.62 (1H, d, J=2.9 Hz, CHO), 5.67~5.81 (2H, m, H-9, H-10), 5.23~5.43 (3H, m, H-3, H-11, H-19), 4.96 (1H, m, H-15), 4.68 (1H, d, J=14.3 Hz, H-27), 4.56 (1H, d, J=14.3 Hz, H-27), 4.43 (1H, m, H-5), 4.01 (1H, s, 7-OH), 3.82 (1H, d, J=5.5 Hz, H-6), 3.68 (1H, dt, J_t=9.9 Hz, J_d=2.9 Hz, H-25), 3.55 (1H, m, H-17), 3.36 (1H, m, H-2), 1.80 (3H, br, H_3-26), 1.55 (3H, br, H_3-29), 1.04 (3H, d, J=7.0 Hz, H_3-28), 0.92 (9H, s, $SiC(CH_3)_3$), 0.13 (3H, s, $SiCH_3$), 0.10 (3H, s, $SiCH_3$), 0.80~2.55 (19H, m, H-12, H_2-13, H_2-16, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31, H_3-32); EI-MS (*m/z*): 670 (M^+), 652, 613, 595, 577, 520; HREI-MS (*m/z*): [M^+]: Calcd for $C_{38}H_{58}O_8Si$, 670.3901; found, 670.3900.

5-Deoxy-5-oxomilbemycin α_{17} (**10**)

A stirred solution of **5** (30.0 mg, 0.05 mmol) and $RhCl(PPh_3)_3$ (50.1 mg, 0.05 mmol) in benzene (10 ml) was refluxed under an argon atmosphere. After stirring for 5 hours, the reaction mixture was cooled down to ambient temperature, filtered through a Celite® pad, washed with EtOAc, and concentrated under reduced pressure. The residue was purified by PLC (Hex - EtOAc = 1 - 2) to give 1.5 mg (5.2%) of **10** and 8.3 mg (27.7%) of recovered **5** as colorless amorphous solids.

10: IR ν_{max} (film) cm^{-1}: 3465, 2960, 2930, 2875, 1735, 1715, 1680; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.55 (1H, m, H-3), 5.90 (1H, dt, J_t=2.4 Hz, J_d=11.0 Hz, H-9), 5.76 (1H, dd, J=14.3, 11.0 Hz, H-10), 5.05~5.55 (4H, m, H-11, H-14, H-15, H-19), 4.75 (2H, m, H_2-27), 4.12 (1H, s, H-6), 3.85 (1H, s, 7-OH), 3.45~3.65 (2H, m, H-2, H-17), 3.05 (1H, dt, J_t=9.2 Hz, J_d=2.6 Hz, H-25), 1.89 (3H, m, H_3-26), 1.02 (3H, d, J=7.0 Hz, H_3-28), 1.00 (3H, t, J=7.7 Hz, H_3-31), 0.83 (3H, d, J=6.2 Hz, H_3-29), 0.70~2.55 (16H, m, H-12, H_2-13, H_2-16, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-30); EI-MS (*m/z*): 526 (M^+), 508, 400, 195, 167; HREI-MS (*m/z*): [M^+]: Calcd for $C_{31}H_{42}O_7$, 526.2931; found, 526.2931.

5-*O*-TBDMS-milbemycin α_{17} (**11**)

A stirred solution of **9** (50 mg, 0.07 mmol) and $RhCl(PPh_3)_3$ (69.1 mg, 0.07 mmol) in benzonitrile (0.5 ml) was heated in an oil bath (165°C preheated) under an argon atmosphere. After stirring for 10 minutes, the reaction mixture was cooled in a water bath, filtered through a Celite® pad, washed with EtOAc, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hex - EtOAc gradient) to give 39.1 mg (81.6%) of **11** as a colorless amorphous solid.

11: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2930, 2850, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.65~5.85 (2H, m, H-9, H-10), 5.03~5.49 (5H, m, H-3, H-11, H-14, H-15, H-19), 4.72 (1H, d, J=14.7 Hz, H-27), 4.58 (1H, d, J=14.7 Hz, H-27), 4.42 (1H, m, H-5), 4.30 (1H, s, 7-OH), 3.82 (1H, d, J=5.5 Hz, H-6), 3.54 (1H, m, H-17), 3.37 (1H, m, H-2), 3.05 (1H, m, H-25), 2.15~2.55 (3H, m, H-12, H_2-16), 1.79 (3H, br, H_3-26), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.93 (9H, s, $SiC(CH_3)_3$), 0.82 (3H, d, J=6.2 Hz, H_3-29), 0.13 (6H, s, $Si(CH_3)_2$), 0.70~2.05 (16H, m, H_2-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-30, H_3-31); EI-MS (*m/z*): 642 (M^+), 585, 567, 400, 382; HREI-MS (*m/z*): [M^+]: Calcd for $C_{37}H_{58}O_7Si$, 642.3952; found, 642.3950.

5-*O*-TBDMS-24-demethylmilbemycin A_4 (**15**)

Using the same procedure described for the preparation of **11**, **15** was prepared from **14** (100 mg, 0.15 mmol) as a colorless amorphous solid in 68.1% yield (65.2 mg, 0.10 mmol).

15: IR ν_{max} (film) cm^{-1}: 3455, 2955, 2935, 2850, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.66~5.82 (2H, m, H-9, H-10), 5.24~5.47 (3H, m, H-3, H-11, H-19), 4.97 (1H, m, H-15), 4.67 (1H, d, J=14.3 Hz, H-27), 4.56 (1H, d, J=14.3 Hz, H-27), 4.42 (1H, m, H-5), 4.15 (1H, s, 7-OH), 3.82 (1H, d, J=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.45 (1H, m, H-25), 3.36 (1H, m, H-2), 2.15~2.55 (4H, m, H-12, H-13, H_2-16), 1.95 (1H, m, H-20), 1.79 (3H, br, H_3-26), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.96 (3H, t,

J=7.3 Hz, H_3-31), 0.93 (9H, s, $Si(CH_3)_3$), 0.13 (3H, s, $SiCH_3$), 0.10 (3H, s, $SiCH_3$), 0.80~1.85 (12H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H_2-24, H_2-30); EI-MS (*m/z*): 642 (M^+), 624, 585, 567, 492; HREI-MS (*m/z*): [M^+]: Calcd for $C_{37}H_{58}O_7Si$, 642.3952; found, 642.3953.

Milbemycin α_{17} (**3**)

To a stirred solution of **11** (39.1 mg, 0.06 mmol) in MeOH (4 ml), *p*-TsOH·H_2O, (39.9 mg, 0.21 mmol) was added at ambient temperature. After stirring for 20 minutes, the reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by PTLC (Hex - EtOAc = 1 - 1) to give 29.9 mg (92.9%) of **3** as a pale yellow amorphous solid.

3: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2915, 2865, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.65~5.89 (2H, m, H-9, H-10), 5.07~5.51 (5H, m, H-3, H-11, H-14, H-15, H-19), 4.78 (2H, m, H_2-27), 4.29 (1H, m, H-5), 4.20 (1H, s, 7-OH), 3.97 (1H, d, J=6.2 Hz, H-6), 3.56 (1H, m, H-17), 3.27 (1H, m, H-2), 3.06 (1H, dt, J_t=9.2 Hz, J_d=2.3 Hz, H-25), 2.20~2.53 (4H, m, H-12, H_2-16, 5-OH), 1.87 (3H, br, H_3-26), 1.02 (3H, d, J=7.7 Hz, H_3-28), 0.99 (3H, t, J=7.7 Hz, H_3-31), 0.83 (3H, d, J=6.6 Hz, H_3-29), 0.70~2.05 (13H, m, H_2-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-30); EI-MS (*m/z*): 528 (M^+), 470, 400, 382, 342, 231, 195, 167; HREI-MS (*m/z*): [M^+]: Calcd for $C_{31}H_{44}O_7$, 528.3087; found, 528.3088.

Milbemycin α_{17} (**3**) was also prepared from 14-formylmilbemycin A_4 (**8**) using a procedure similar to that described for the preparation of **10** in 5.6% yield (7.4% of **8** was recovered, see Table 1).

24-Demethylmilbemycin A_4 (**4**)

Using the same procedure described for the preparation of **3**, **4** was prepared from **15** (60 mg, 0.09 mmol) as a colorless amorphous solid in 95.3% yield (47 mg, 0.09 mmol).

4: IR ν_{max} (film) cm^{-1}: 3465, 2965, 2940, 2920, 2865, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.65~5.87 (2H, m, H-9, H-10), 5.27~5.49 (3H, m, H-3, H-11, H-19), 4.98 (1H, m, H-15), 4.68 (2H, m, H_2-27), 4.29 (1H, m, H-5), 4.13 (1H, s, 7-OH), 3.96 (1H, d, J=6.2 Hz, H-6), 3.58 (1H, m, H-17), 3.42 (1H, m, H-25), 3.26 (1H, m, H-2), 2.32~2.55 (2H, m, H-12, 5-OH), 2.10~2.30 (3H, m, H-13, H_2-16), 1.87 (3H, br, H_3-26), 1.53 (3H, br, H_3-29), 0.99 (3H, d, J=6.6 Hz, H_3-28), 0.97 (3H, t, J=7.3 Hz, H_3-31), 0.78~2.08 (13H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H_2-24, H_2-30); EI-MS (*m/z*): 528 (M^+), 400, 382, 250, 231, 181; HREI-MS (*m/z*): [M^+]: Calcd for $C_{31}H_{44}O_7$, 528.3087; found, 528.3088.

Acaricidal Activity against Two-spotted Spider Mites (*Tetranychus urticae*)

The primary leaves of cowpea plants (*Vigna sinensis Savi* species) were infected with the organic phosphate-sensitive two-spotted spider mites (*Tetranychus urticae*). One day after the infection, the infested plants were sprayed (Mizuho rotary sprayer) with 7 ml of a *p*-xylene solution containing the test compound at concentrations ranging from 1 to 10 ppm at a rate of 3.5 mg of test solution per 1 cm^2 of leaf. The plants were assessed after 3 days by examining the adult mites under a binocular microscope to determine the numbers of living and dead individuals. Two plants were used for each concentration and each test compound. The plants were kept in greenhouse compartments at 25°C during the test. The results are reported in Table 2.

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