Synthesis of 27-Oxo, 27-Hydroxymilbemycins A₃ and A₄ and Novel 27-Alkoxymilbemycins A₃ and A₄ from Milbemycins A₃ and A₄ and Their Acaricidal Activities

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27-Oxomilbemycins A₃ and A₄ and 27-hydroxymilbemycins A₃ and A₄ were identified as metabolites in soil metabolism studies of milbemycins A₃ and A₄. Chemical derivation methods were developed to synthesize 27-oxomilbemycins A₃ and A₄ and 27-hydroxymilbemycins A₃ and A₄ from milbemycins A₃ and A₄. In addition, 27-alkoxymilbemycin derivatives were also synthesized from the same precursors. Some of the synthesized compounds displayed satisfactory acaricidal activity against the organophosphorus-sensitive two-spotted spider mite (Tetranychus urticae), but did not have superior activity to corresponding milbemycins A₃ and A₄.

Key words: milbemycin; acaricide

Milbemycins⁴⁻⁷ are sixteen-membered ring macrolides that have been isolated from Streptomyces hygroscopicus. They exhibit notable activities as acaricides, insecticides and anthelmintics. Among them, milbemectin⁸ [a mixture of milbemycins A₃ (1a) and A₄ (1b) (Fig. 1)] has been developed as an agricultural acaricide. Abamectin⁹ [a mixture of avermectins ¹⁰ B₁a (2a) and B₁b (2b) (Fig. 1)] has similar structural and biological features, and has been developed as an agricultural acaricide and an insecticide. Ivermectin¹¹ [a mixture of 22, 23-dihydroavermectin B₁a (3a) and 22, 23-dihydroavermectin B₁b (3b) (Fig. 1)], which was derived from abamectin, has been developed as an anthelmintic for livestock.

Milbemycins have been reported to be metabolized to various compounds in the environment.⁶ 27-Oxomilbemycins A₃ (4a) and A₄ (4b) (Fig. 1) and 27-hydroxymilbemycins A₃ (5a, 5’a) and A₄ (5b, 5’b) (equilibrium mixtures) (Scheme 1) have been identified as metabolites in soil of milbemycins A₃ (1a) and A₄ (1b).⁶ As these soil metabolites are required as reference materials for further study of environmental chemistry, methods to prepare them by chemical derivation from milbemycins A₃ (1a) and A₄ (1b) have become necessary.

The remarkable biological properties of milbemycins and avermectins have stimulated the interest of many research groups. Enormous effort has been made to produce semi-synthetic congeners¹²⁻¹⁶ with improved biological activities, and to search for new analogs from nature.¹⁷⁻²⁰ However, the 27-alkoxymilbemycins have only been reported as a natural product²³ in one study, and no study has been made on how the introduction of an alkoxy moiety to the C-27 position and its stereochemistry would influence biological activities. To elucidate these points, we developed a synthetic method to prepare 27-alkoxymilbemycins and then assessed the biological activities of the samples prepared by this method.

We report here the synthetic methods for 27-oxomilbemycins A₃ (4a) and A₄ (4b), 27-hydroxymilbemycins A₃ (5a, 5’a) and A₄ (5b, 5’b) and 27-alkoxymilbemycins A₃ and A₄ from milbemycins A₃ (1a) and A₄ (1b), as well as the acaricidal activities of these milbemycins.

Results and Discussion

Chemistry

27-Oxomilbemycins A₃ (4a) and A₄ (4b) were synthesized by following method (Scheme 1). The Merck chemists reported oxidizing the C-27 position of the milbemycin framework to a lactone moiety with pyridinium dichromate²⁵,²⁶ in N,N-dimethylformamide. Applying the conditions to 5-O-tert-butyl(dimethyl)silyl(TBDMs)-milbemycin A₄ (6b)²⁷ gave 5-O-TBDMs-27-oxomilbemycin A₄ (7b) in a poor yield (10%). After unsuccessful trials, oxidation of

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the C-27 position was best accomplished by using chromium (VI) oxide (CrO$_3$) in pyridine$^{28,29}$ to afford 27-oxo compounds 7a and 7b. Subsequent deprotection of the 5-O-TBDMS groups with p-toluenesulfonic acid monohydrate (p-TsOH·H$_2$O) in methanol (MeOH)$^{25,26}$ gave 27-oxomilbemycins A$_3$ (4a) and A$_4$ (4b) in good yields. All spectral data for synthesized 4a and 4b are in agreement with those of the reference materials.$^8$

The Merck chemists reported directly introducing a hydroxy moiety to the C-27 position of the milbemycin framework by means of oxidation with t-butyl peroxybenzoate and copper (I) chloride, and subsequent deprotection with acetic acid.$^{30}$ Applying these conditions to 6b gave 27-hydroxymilbemycin A$_4$ (an equilibrium mixture of 5b and 5b') in a poor yield (only 9%). Hence, we attempted selective half-reduction of the C-27 lactone moiety of 5-O-TBDMS-27-oxomilbemycins A$_3$ (7a) and A$_4$ (7b) into a lactol (Scheme 1). Reduction with diisobutylaluminum hydride (DIBAL-H) in toluene afforded the desired products in moderate yields. The products were obtained as equilibrium mixtures in the hemiacetal form (8a and 8b) and cleaved aldehyde form (8a' and 8b'). Deprotection of the 5-O-TBDMS groups with p-TsOH·H$_2$O in tetrahydrofuran (THF) and water afforded 27-hydroxymilbemycins A$_3$ (5a and 5a') and A$_4$ (5b and 5b') in good yields (Scheme 1). All spectral data for synthesized 5a and 5a' and 5b and 5b' are in agreement with those of the reference materials.$^6$

To better characterize the role of the C-27 position in biological activities, the preparation method for 27-alkoxymilbemycins was examined (Scheme 1). The treatment of 5-O-TBDMS-27-hydroxymilbemycins A$_3$ (8a) and A$_4$ (8b) with a variety of alcohols in the presence of p-TsOH·H$_2$O afforded 27-alkoxymilbemycins A$_3$ (9a and 9a') and A$_4$ (9b, 9b', 10b, 10b', 11b and 11b') as mixtures of diastereomers arising from the asymmetric center at the C-27 position. Deprotection of the C-5 hydroxy group was concomitantly achieved in a one-pot reaction. These diastereomixtures were separable into each isomer by silica gel purification. The yields of each derivative are summarized in Table 1. The stereochemistry of the C-27 position of each derivative was then determined. Nuclear Overhauser effect (NOE) observation between C(2)-H and C(27)-H of each derivative was then determined. Nuclear Overhauser effect (NOE) observation (Fig. 1). All spectral data for synthesized 4a and 4b are in agreement with those of the reference materials.$^6$

The stereochemistry of the isomers of the other derivatives was determined by comparing their $^1$H-NMR spectra with those of 27-methoxymilbemycin A$_3$ (9a) and 27β-methoxymilbemycin A$_3$ (9a').

**Acaricidal activities**

The synthesized 27-oxomilbemycins A$_3$ (4a), A$_4$ (4b), 27-hydroxymilbemycins A$_3$ (5a, 5a'), A$_4$ (5b, 5b') and 27-alkoxymilbemycin derivatives (9a, 9a’, 9b, 9b’, 10b, 10b’, 11b, and 11b’) were studied to assess their acaricidal activities against the two-spotted spider mite (*Tetranychus urticae*). The results are listed in Table 2. Oxidation of the C-27 position to a lactone moiety drastically reduced the acaricidal activity (4a and 4b). On the other hand, the derivatives with hydroxy and alkoxy moieties at the C-27 position retained their acaricidal activity (5a, 5a’, 5b, 5b’, 9a, 9a’, 9b, 9b’, 10b, 10b’, 11b, and 11b’). These observations may suggest that the sp$^3$ structure of the C-27 position was essential for high acaricidal activity. Concerning the stereochemistry at the C-27 position, the β-configuration was generally associated with better acaricidal activity than the α-configuration. Concerning the steric bulkiness of the substituents of the C-27 alkoxoy moiety at the C-27 position, smaller ones were slightly better for activity. It can be assumed that the lower acaricidal activity of the 27-hydroxy derivatives (5a, 5a’, 5b and 5b’) than the corresponding 27-methoxy derivatives (9a,
Reagents: (a) CrO₃, pyridine; 37% for 7a, 40% for 7b; (b) p-TsOH・H₂O, MeOH; 91% for 4a, 94% for 4b; (c) DIBAL-H, cyclohexane-toluene; 34% for 8a and 8a’; 41% for 8b and 8b’; (d) p-TsOH・H₂O, THF-H₂O; 84% for 5a and 5a’; 90% for 5b and 5b’; (e) R’OH, p-TsOH・H₂O, see Table 1.

Table 1. Yields of 27-Alkoxymilbemycins

<table>
<thead>
<tr>
<th>R</th>
<th>R’</th>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td>Me</td>
<td>8a, 8a’</td>
<td>9a (R = Me, R’ = Me) 64% and 9a’ (R = Me, R’ = Me) 17%</td>
</tr>
<tr>
<td>Et</td>
<td>Me</td>
<td>8b, 8b’</td>
<td>9b (R = Et, R’ = Me) 58% and 9b’ (R = Et, R’ = Me) 19%</td>
</tr>
<tr>
<td>Et</td>
<td>Et</td>
<td>8b, 8b’</td>
<td>10b (R = Et, R’ = Et) 57% and 10b’ (R = Et, R’ = Et) 12%</td>
</tr>
<tr>
<td>Et</td>
<td>iPr</td>
<td>8b, 8b’</td>
<td>11b (R = Et, R’ = iPr) 53% and 11b’ (R = Et, R’ = iPr) 15%</td>
</tr>
</tbody>
</table>

9a’, 9b and 9b’) was due to inhibition in their transportation to the target site by the higher polarity of 5a, 5a’, 5b and 5b’. However, the activity of the newly synthesized compounds reported here was inferior that of milbemycin A₃ (1b). In short, the introduction of hydroxy and alkoxy moieties to the C-27 position was possible, but it was not essential to improve the acaricidal activities.

Conclusion

In conclusion, we established a method to prepare 27-oxo and 27-hydroxymilbemycins A₃ and A₄ (4a, 4b, 5a, 5a’, 5b and 5b’) from milbemycin A₃ (1a) and A₄ (1b). 27-Alkoxymilbemycin derivatives (9a, 9a’, 9b, 9b’, 10b, 10b’, 11b, and 11b’) were also synthesized and their acaricidal activities were assessed. It was clarified that the introduction of hydroxy and alkoxy moieties to the C-27 position of milbemycins
was possible but not essential to improve the acaricidal activities.

**Experimental**

NMR spectra were measured with a Varian Gemini-200 FT NMR spectrometer at 200 MHz, chemical shifts (δ) being expressed in parts per million relative to the internal standard, tetramethylsilane. Mass spectra were measured with a Fisons Instruments VG Autospec, and IR spectra were measured with a Perkin Elmer 1600 series FT IR instrument.

5-O-TBDM-27-oxamibemycin A₃ (7a). To stirred pyridine (280 ml) in an ice bath was slowly added CrO₃ (26.2 g, 0.26 mol), while maintaining the temperature at under 10°C. To this solution was added a solution of 5-O-TBDM-milbemycin A₃ (6a; 19.4 g, 0.03 mol) in pyridine (200 ml). After stirring for 1 hour, the ice bath was removed, and stirring was continued at ambient temperature. After 4 days, the reaction mixture was poured into a mixture of 1N hydrochloric acid and ethyl acetate (EtOAc). After stirring for 15 minutes, the insoluble material was filtered off with Celite®, and the filtrate was extracted with EtOAc. The extract was successively washed twice with 1N hydrochloric acid, water and brine, dried over magnesium sulfate (MgSO₄), and evaporated in vacuo to give 7.30 g (37%).

The residue was purified by silica gel chromatography [n-hexane (Hex)-EtOAc gradient] to give 7.30 g (37%) of 7a and 2.78 g (14%) of recovered 6a as pale yellow amorphous solids.

7a: IR νmax (film) cm⁻¹: 3470, 2955, 2925, 2880, 2855, 1755, 1710, 1640; ¹H-NMR (200 MHz, CDCl₃) δ: 7.26 (1H, dd, J = 15.3, 11.4 Hz, H-10), 6.52 (1H, d, J = 11.4 Hz, H-9), 5.84 (1H, dd, J = 15.3, 9.6 Hz, H-11), 5.63 (1H, m, H-3), 5.52 (1H, m, H-19), 5.02 (1H, m, H-15), 4.66 (1H, s, -7-OH), 4.37 (1H, d, J = 5.7 Hz, H-5), 4.20 (1H, d, J = 5.7 Hz, H-6), 3.69 (1H, t, J = 2.7 Hz, H-2), 3.60 (1H, m, H-17), 3.27 (1H, dd, J = 9.5, 6.2 Hz, H-25), 2.60 (1H, m, H-12), 1.89 (3H, t, J = 2.0 Hz, H-26), 1.55 (3H, br, H-29), 1.14 (3H, d, J = 6.2 Hz, H-31), 1.02 (3H, d, J = 6.7 Hz, H-28), 0.86 (9H, s, (CH₃)₃CSi), 0.82 (3H, d, J = 8.1 Hz, H₃-30), 0.15 (3H, s, CH₃Si), 0.10 (3H, s, CH₃Si), 0.70-0.240 (13H, m, H-13, H-16, H-18, H-20, H-22, H-23, H-24); EI-MS (m/z): 656 (M⁺), 599, 581; HREI-MS (m/z): [M⁺]: calcd. for C₅₈H₅₆O₈Si, 650.3744; found, 656.3744.

27-Oxamibemycin A₄ (4a). To a stirred solution of 1.00 g (1.52 mmol) of 5-O-TBDM-27-oxamibemycin A₃ (7a) in MeOH (20 ml) was added 0.87 g (45.7 mmol) of p-TsOH-H₂O at ambient temperature. After stirring for 2 hours, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over MgSO₄, and evaporated in vacuo.

The residue was purified by silica gel chromatography (Hex-EtOAc gradient) to give 0.75 g (91%) of 4a as a pale yellow amorphous solid.

4a: IR νmax (film) cm⁻¹: 3460, 2965, 2920, 2870, 1730, 1710, 1640; ¹H-NMR (200 MHz, CDCl₃) δ: 7.21 (1H, dd, J = 15.0, 11.4 Hz, H-10), 6.58 (1H, d, J = 11.4 Hz, H-9), 5.89 (1H, dd, J = 15.0, 9.9 Hz, H-11), 5.72 (1H, br, H-3), 5.50 (1H, m, H-19), 5.02 (1H, m, H-15), 4.67 (1H, s, -7-OH), 4.48 (1H, m, H-5), 4.30 (1H, d, J = 5.5 Hz, H-6), 3.48-3.65 (2H, m, H-2, H-17), 3.27 (1H, dd, J = 9.5, 6.6 Hz, H-25), 2.65 (1H, m, H-12), 2.15-2.35 (4H, m, H-13, H-16, 5-OH), 1.95 (3H, br, H-26), 1.54 (3H, br, H-29), 1.15 (3H, d, J = 6.2 Hz, H-28), 1.03 (3H, d, J = 6.6 Hz, H-31), 0.84 (3H, d, J = 6.6 Hz, H-30), 0.80-2.00 (10H, m, H-13, H-18, H-20, H-22, H-23, H-24); EI-MS (m/z): 542 (M⁺), 524; HREI-MS (m/z): [M⁺]: calcd. for C₃₇H₄₂O₁₂Si, 542.2880; found, 542.2881.

27-Oxamibemycin A₄ (4b). Using the same procedure as that described for the preparation of 4a, 1.00 g (1.49 mol) of 7b was treated with 0.85 g (4.48 mol) of p-TsOH·H₂O in MeOH (20 ml) to give 0.78 g (94%) of 4b as a pale yellow amorphous solid.

4b: IR νmax (film) cm⁻¹: 3465, 2955, 2920, 2905, 1745, 1710, 1640; ¹H-NMR (200 MHz, CDCl₃) δ: 7.27 (1H, m, H-10), 6.59 (1H, d, J = 11.4 Hz, H-9), 5.92 (1H, dd, J = 9.9, 5.1 Hz, H-11), 5.73 (1H, br, H-3), 5.49 (1H, m, H-19), 5.00 (1H, m, H-15), 4.67 (1H, s, -7-OH), 4.49 (1H, m, H-5), 4.30 (1H, d, J = 5.5 Hz, H-6), 3.50-3.70 (2H, m, H-2, H-17), 3.07 (1H, m, H-25), 2.55-2.70 (1H, m, H-12), 1.96 (3H,
br, H2, 26), 1.54 (3H, br, H2-29), 1.04 (3H, d, J = 6.6 Hz, H2-28), 1.01 (3H, t, J = 7.3 Hz, H3-32), 0.83 (3H, d, J = 6.2 Hz, H3-30), 0.82–2.35 (16H, m, H2-13, H2-16, H2-18, H2-20, H2-22, H2-23, H-24, H3-21, 5-OH); EI-MS (m/z): 556 (M+), 538, 520; HREI-MS (m/z): [M]+: calcd. for C32H42O8S, 556.3036; found, 556.3037.

5-O-TBDMST-27-hydroxyamilbemycin A2 (8a and 8a'). To a stirred solution of 100 mg (0.15 mmol) of 5-O-TBDMST-27-oxoamilbemycin A2 (7a) in toluene (4 ml) was added dropwise a 0.9 ml (0.9 mmol) of a 1M solution of DIBAL-H in cyclohexane in a nitrogen atmosphere while cooling with a dry ice-acetone bath. After stirring for 1 hour, an additional 0.9 ml (0.9 mmol) of the 1M solution of DIBAL-H in cyclohexane was added dropwise. After stirring for another 1 hour, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over MgSO4 and evaporated in vacuo. The residue was purified by preparative TLC to give 34.2 mg (34%) of 8a as an equilibrium mixture with 8a' (8a:8a' = 1:3, pale yellow amorphous solid).

Equilibrium mixture of 8a and 8a': IR νmax (film) cm⁻¹: 3455, 2955, 2925, 2880, 2850, 1705, 1685; 1H-NMR (200 MHz, CDCl3) δ: 10.05 (0.75H, d, J = 1.8 Hz, CHO), 6.95 (0.75H, dd, J = 13.9, 11.4 Hz), 6.85 (0.75H, dd, J = 11.4, 1.8 Hz), 5.86–6.02 (0.5H, m), 5.72 (0.75H, dd, J = 13.9, 9.5 Hz), 5.25–5.51 (2.5H, m), 5.00 (0.25H, m), 4.81 (0.75H, m), 4.49 (1H, br), 4.20 (0.25H, m), 4.08 (0.25H, s), 3.97 (0.75H, m), 3.72 (0.75H, d, J = 4.0 Hz), 3.55(1H, m), 3.15–3.31 (2H, m), 2.55 (1H, m), 2.11–2.33 (3H, m), 1.77 (3H, br), 1.60 (3H, br), 0.93 (2.25H, s), 0.92 (6.75H, s), 0.83 (0.75H, d, J = 6.6 Hz), 0.82 (2.25H, d, J = 6.6 Hz), 0.17 (0.75H, s), 0.15 (3H, s), 0.11 (2.25H, s), 0.60–2.70 (17H, m); EI-MS (m/z): 658 (M+), 640, 610, 583; HREI-MS (m/z): [M]+: calcd. for C37H58O8Si, 672.4057; found, 672.4057.

27-Hydroxymilbemycin A2 (5a and 5a'). To a stirred solution of 290.0 mg (0.44 mmol) of 5-O-TBDMST-27-hydroxyamilbemycin A2 (8a and 8a') in THF (9 ml) and water (3 ml) was added 251.5 mg (1.32 mmol) of p-TsOH·H2O at ambient temperature. After stirring overnight, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over MgSO4 and evaporated in vacuo. The residue was purified by preparative TLC to give 202.4 mg (84%) of 5a as an equilibrium mixture with 5a' (5a:5a' = 1:1, pale yellow amorphous solid).

Equilibrium mixture of 5a and 5a': IR νmax (film) cm⁻¹: 3440, 2970, 2920, 2870, 1710, 1680, 1650, 1620, 1575; 1H-NMR (200 MHz, CDCl3) δ: 10.09 (0.5H, s, CHO), 7.59 (0.5H, d, J = 12.1 Hz), 6.72 (0.5H, dd, J = 14.7, 12.1 Hz), 5.80–6.27 (1.5H, m), 5.72 (0.5H, s), 5.18–5.58 (2.5H, m), 4.82–5.10 (1H, m), 4.15–4.45 (2H, m), 4.05 (1H, s), 3.85 (0.5H, m), 3.74 (0.5H, m), 3.47–3.70 (1.5H, m), 3.10–3.35 (1H, m), 1.87 (3H, br), 1.58 (3H, br), 0.83 (1.5H, d, J = 6.2 Hz), 0.81 (1.5H, d, J = 6.6 Hz), 0.70–2.70 (22H, m); EI-MS (m/z): 544 (M+), 526, 508, 490, 416, 398; HREI-MS (m/z): [M]+: calcd. for C38H60O8Si, 544.3036; found, 544.3035.

27-O-TBDMST-27-hydroxyamilbemycin A2 (8b and 8b'). Using the same procedure as that described for the preparation of the equilibrium mixture of 5a and 5a', 500.0 mg (0.74 mmol) of 8b and 8b' was treated with 424.6 mg (2.23 mmol) of p-TsOH·H2O in THF (15 ml) and water (5 ml) to give 372.8 mg (90%) of 5b as an equilibrium mixture with 5b' (5b:5b' = 1:1, colorless amorphous solid).

Equilibrium mixture of 5b and 5b': IR νmax (film) cm⁻¹: 3440, 2955, 2920, 2865, 1710, 1680, 1620; 1H-NMR (200 MHz, CDCl3) δ: 10.09 (0.5H, s, CHO), 7.59 (0.5H, d, J = 11.7 Hz), 6.75 (0.5H, dd, J = 14.6, 12.1 Hz), 5.89–6.30 (1.5H, m), 5.72 (0.5H, br), 5.20–5.70 (2.5H, m), 4.83–5.01 (1H, m), 4.15–4.42 (2H, m), 4.04 (0.5H, s), 3.84 (0.5H, d, J = 4.0 Hz), 3.73 (0.5H, m), 3.52–3.64 (1H, m), 2.90–3.25 (1.5H, m), 1.89 (3H, br), 1.62 (3H, br), 0.95 (3H, m), 0.83 (1.5H, d, J = 6.2 Hz), 0.81 (1.5H, d, J = 6.6 Hz), 0.63–2.80 (21H, m); EI-MS (m/z): 558 (M+), 540, 522, 504; HREI-MS (m/z): [M]+: calcd. for C39H62O8Si, 558.3193; found, 558.3192.

27α-Methoxyamilbemycin A3 (9a) and 27β-
methoxymilbemycin A1 (9a'). To a stirred solution of 34.0 mg (0.05 mmol) of 5-O-TBDMs-27-hydroxymilbemycin A1 (8a and 8a') in MeOH (1 ml) was added 30.0 mg (0.16 mmol) of p-TsOH-H2O at ambient temperature. After stirring for 30 min, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over MgSO4 and evaporated in vacuo. The residue was purified by preparative TLC to give 18.5 mg (64%) of 9a (more polar) and 4.9 mg (17%) of 9a' (less polar) as pale yellow amorphous solids.

9a: IR νmax (film) cm⁻¹: 3465, 2960, 2920, 2870, 1710; ¹H-NMR (200 MHz, CDCl3) δ: 5.94–6.13 (2H, m, H-9, H-10), 5.58 (1H, d, J=1.5 Hz, H-27), 5.30–5.51 (3H, m, H-3, H-11, H-19), 5.00 (1H, m, H-15), 4.30 (1H, dd, J=7.7, 6.2 Hz, H-5), 4.23 (1H, d, J=6.2 Hz, H-6), 4.10 (1H, s, 7-OH), 3.47–3.62 (1H, m, H-17), 3.50 (3H, s, CH3O), 3.26 (1H, dd, J=9.5, 6.4 Hz, H-25), 3.17 (1H, m, H-2), 2.45–2.50 (1H, m, H-12), 2.33 (1H, br, J=7.5 Hz, 5-OH), 1.87 (3H, br, H3-26), 1.54 (3H, br, H3-29), 1.02 (3H, d, J=6.6 Hz, H3-28), 0.89 (3H, t, J=7.3 Hz, H3-30), 0.83 (3H, d, J=6.2 Hz, H3-30), 0.78–2.05 (12H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H3-31); EI-MS (m/z): 572 (M⁺), 540, 504; HREI-MS (m/z): [M⁺]: calcd. for C33H48O8, 572.3349; found, 572.3348.

9b': IR νmax (film) cm⁻¹: 3475, 2955, 2915, 2870, 1710; ¹H-NMR (200 MHz, CDCl3) δ: 5.96–6.13 (2H, m, H-9, H-10), 5.60–5.65 (2H, br, H-3, H-27), 5.35–5.60 (2H, m, H-13, H-19), 4.98 (1H, t, J=7.7 Hz, H-15), 4.28 (1H, dd, J=5.9, 3.3 Hz, H-5), 4.22 (1H, s, 7-OH), 4.08 (1H, d, J=5.9 Hz, H-6), 3.69 (1H, t, J=2.6 Hz, H-2), 3.58 (1H, m, H-17), 3.47 (3H, s, CH3O), 3.09 (1H, m, H-25), 3.02 (1H, d, J=3.3 Hz, 5-OH), 2.48 (1H, m, H-12), 2.15–2.30 (3H, m, H-13, H-19), 1.93 (3H, br, H2-26), 1.52 (3H, br, H2-29), 1.01 (3H, d, J=7.0 Hz, H-25), 1.00 (1H, t, J=7.7 Hz, H-32), 0.82 (3H, d, J=6.2 Hz, H3-30), 0.70–2.05 (12H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H3-31); EI-MS (m/z): 572 (M⁺), 554, 540, 504; HREI-MS (m/z): [M⁺]: calcd. for C33H48O8, 572.3349; found, 572.3350.

27α-Ethoxymilbemycin A1 (9b) and 27β-ethoxymilbemycin A1 (9b'). Using the same procedure as that described for the preparation of 9a and 9a', 50.0 mg (0.07 mmol) of 5-O-TBDMs-27-hydroxymilbemycin A1 (8b and 8b') was treated with 42.0 mg (0.22 mmol) of p-TsOH-H2O in ethanol (EtOH, 1 ml) to give 24.8 mg (57%) of 10b (more polar) and 5.4 mg (12%) of 10b' (less polar) as pale yellow amorphous solids.

10b: IR νmax (film) cm⁻¹: 3470, 2960, 2915, 2870, 1710; ¹H-NMR (200 MHz, CDCl3) δ: 5.95–6.15 (2H, m, H-9, H-10), 5.69 (1H, d, J=1.5 Hz, H-27), 5.30–5.53 (3H, m, H-3, H-11, H-19), 4.98 (1H, m, H-15), 4.30 (1H, dd, J=7.3, 6.2 Hz, H-5), 4.25 (1H, d, J=6.2 Hz, H-6), 4.02 (1H, s, 7-OH), 3.80–3.95 (1H, m, H-32), 3.75 (1H, m, H-5), 3.02 (1H, d, J=9.2 Hz, H3-30), 0.82–2.05 (10H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H3-31); EI-MS (m/z): 586 (M⁺), 540, 504; HREI-MS (m/z): [M⁺]: calcd. for C34H50O9, 586.3506; found, 586.3507.
27α-Isopropoxymilbemycin A₄ (11b) and 27β-isopropoxymilbemycin A₄ (11b'). Using the same procedure as that described for the preparation of 9a and 9a', 50.0 mg (0.07 mmol) of 5-O-TBDMS-27-hydroxymilbemycin A₄ (8b and 8b') was treated with 42.0 mg (0.22 mmol) of p-TsOH·H₂O in 2-propanol (iPrOH, 1 ml) to give 23.6 mg (53% of 11b) of more polar and 6.5 mg (15%) of 11b' (less polar) as pale yellow amorphous solids.

11b): IR νₑₑₑ (film) cm⁻¹: 3460, 2970, 2915, 2870, 1710; ¹H-NMR (200 MHz, CDCl₃) δ: 5.97–6.12 (2H, m, H-9, H-10), 5.74 (1H, s, H-27), 5.63 (1H, br, H-3), 5.34–5.56 (2H, m, H-11, H-19), 4.98 (1H, m, H-15), 4.28 (1H, dd, J = 5.9, 3.3 Hz, H-5), 4.23 (1H, s, 7-OH), 4.06 (1H, d, J = 5.9 Hz, H-6), 3.54–3.85 (4H, m, H-2-20, H-2-22, H-2-23, H-24, H-2-31); EI-MS (m/z): 600 (M+), 540, 522, 504; HREI-MS (m/z): [M⁺]: calcd. for C₃₅H₅₂O₈, 600.3662; found, 600.3661.

Acaricidal activity against the two-spotted spider mite (Tetranychus urticae). The primary leaves of cowpea plants (Vigna sinensis Savi species) were infected with the organic phosphate-sensitive two-spotted spider mite (Tetranychus urticae). One day after infection, the infested plants were sprayed (Mizuho rotary sprayer) with 7 ml of a solution containing a test compound at a concentration ranging from 1 to 10 ppm at a rate of 3.5 mg of the test solution per 1 cm² 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 during the test in green-house compartments at 25°C. The results are reported in Table 2.

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References and Notes


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