Synthesis of 5-Keto-5-oxime Derivatives of Milbemycins and Their Activities against Microfilariae

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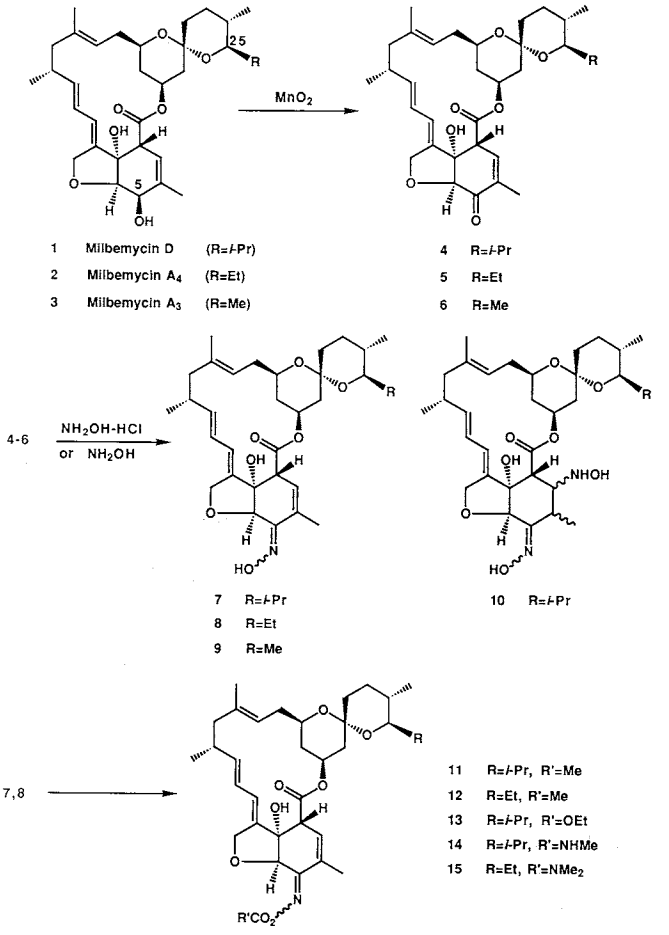
Starting from milbemycin D (1), milbemycin A_4 (2) and milbemycin A_3 (3), a series of 5-keto-5-oxime derivatives were synthesized by selective oximation at the α,β-conjugated carbonyl function of the 5-ketomilbemycins (4–6). The activities of the synthesized compounds were studied in dogs naturally infested with microfilariae of *Dirofilaria immitis*. The 5-keto-5-oximes of milbemycin D (7), A_4 (8) and A_3 (9) had quite high efficacy to control the microfilariae and more potency than their parents, while the 5-*O*-acyl oximes (11–15) also exhibited high activity.

Milbemycins, which are known to have a potent and broad spectrum of anthelmintic, acaricidal and insecticidal activities, are sixteen-membered-ring macrolides isolated from *Streptomyces hygroscopicus*.[1,2] Among them, milbemycin D (**1**) is now in commercial use for the treatment and control parasites in dogs, and a 7:3 mixture of milbemycin A_4 (**2**) and milbemycin A_3 (**3**) has recently been brought to the market as an agricultural miticide. During a study on the chemistry of milbemycins, we have been especially interested in the chemical transformation of the allylic hydroxy group at the 5-position. Starting from milbemycin D, A_4 and A_3, we synthesized a series of 5-keto-5-oxime derivatives and tested their activities against microfilariae. In this paper, we report details of the synthesis and the activities of 5-keto-5-oxime derivatives of milbemycins.[3-5]

The synthesis of the 5-keto-5-oxime derivatives is shown in Scheme 1. The 5-keto intermediates of milbemycin A_3 (**6**) and A_4 (**5**) were also isolated from a culture broth of *Streptomyces hygroscopicus*, and were named milbemycin J and K, respectively.[1] Milbemycin D has been reported to be transformed to the corresponding keto derivative by the general oxidation method.[6] We adopted active manganese dioxide for the oxidation of milbemycins (**1**–**3**) to afford 5-ketomilbemycins (**4**–**6**) in 70–80% yields. By other oxidation reactions such as Jones oxidation,[7] Swern oxidation[8] and Collins oxidation,[9] 5-ketomilbemycins were also obtained in rather low yields (50–60%). Oximation of α,β-unsaturated ketones (**4**–**6**) with hydroxylamine hydrochloride (hydroxylamine hydrochloride in dioxane–methanol–water) gave 5-ketomilbemycin-5-oximes (**7**–**9**) exclusively in high yields. On the other hand, the use of free hydroxylamine (hydroxylamine hydrochloride-sodium acetate in methanol) in the reaction of **4** afforded an undesired bis-adduct (**10**, 34.9%) in addition to **7** (22.8%). It is important, therefore, to use the hydrochloride salt of hydroxylamine as a weaker nucleophile for selective oximation at the 5-position of the α,β-conjugated carbonyl group in the intermediates (**4**–**6**). The obtained oximes (**7** and **8**)



Scheme 1.

were further converted to 5-*O*-acetyloximes (**11** and **12**), 5-*O*-ethoxycarbonyloxime (**13**), 5-*O*-methylcarbamoyloxime (**14**) and 5-*O*-dimethylcarbamoyloxime (**15**).

The activities of the synthesized compounds were studied in dogs naturally infested with microfilariae of *Dirofilaria immitis*,[10] the results being summarized in Table I. Parent 5-hydroxy milbemycin D (**1**) and A_4 (**2**), excepting A_3 (**3**), showed high activity, whereas 5-ketomilbemycin A_4 (**5**) exhibited only moderate activity. To our delight, the 5-keto-5-oxime derivatives exhibited high activity. Especially, the 5-keto-5-oximes of milbemycin D (**7**) and A_4 (**8**), and 5-ketomilbemycin A_4 5-*O*-dimethylcarbamoyloxime (**15**) had quite high efficacy for controlling microfilariae. Their activities were superior to that of milbemycin D (**1**), which was the most active compound among parent 5-hydroxyl milbemycins.[11] 5-Keto-5-oxime of milbemycin A_3 (**9**) also had high activity against microfilariae and more potency than the parent.

In the structure-activity relationship at the 5-position of milbemycins, the hydroxyimino function turned out not only to act as a bioisostere of the hydroxyl function, but to potentiate the activity against microfilariae. Recently, a mixture of 5-keto-5-oximes of milbemycin A_4 and A_3 ($A_4 : A_3 = 80 : 20$) was launched as a parasiticide for dogs in U.S.A.

Table I. Effect of Milbemycin Derivatives against Microfilariae of *Dirofilaria immitis* in Naturally Infested Dogs[a]

Compd.	Mil.[b]	Substituent at the 5-position	Dose[c] (mg/kg)	Reduction (%)[d] 1 day	1 week	2 weeks
1	D	β-OH	0.05	99.6	90.1	92.5
2	A_4	β-OH	0.05	94.1	74.3	86.2
3	A_3	β-OH	0.05	40.9	22.6	11.7
5	A_4	=O	0.1	12.2	42.6	45.4
7	D	=NOH	0.05	69.0	95.0	95.5
8	A_4	=NOH	0.05	87.8	93.2	93.2
9	A_3	=NOH	0.05	89.3	85.2	67.0
	A_{4+3}[e]	=NOH	0.05	88.8	89.8	90.5
11	D	=NOCOMe	0.05	36.0	63.9	79.9
12	A_4	=NOCOMe	0.05	89.9	89.9	85.3
13	D	$=NOCO_2Et$	0.1	93.2	98.8	98.0
14	D	=NOCONHMe	0.1	56.8	61.5	81.6
15	A_4	$=NOCONMe_2$	0.05	93.0	93.4	97.7

[a] The dogs were obtained from Animal Protection Center of Kanagawa Prefecture.
[b] Milbemycin.
[c] Orally administrated (*p.o.*).
[d] Percentage reduction of microfilariae found in blood samples = ((B − A)/B) × 100%: B = number of microfilariae before treatment; A = number of microfilariae after treatment.
[e] A mixture of milbemycin A_4 and A_3 ($A_4 : A_3 = 80 : 20$).

Experimental

IR spectra were recorded on a Perkin Elmer 1600 Series FT IR spectrometer. ^{1}H-NMR spectra were measured on a JOEL JNM-GX 270 FT NMR spectrometer with tetramethylsilane as an internal standard. MS spectra were measured on a JEOL JMS-D 300 spectrometer. Column chromatography was performed on silica gel (60 Merck, 230–400 mesh or Wakogel C-100, 40–100 mesh). Preparative TLC was performed on silica gel (Merck 60 PF_{254}) of 0.5 mm thickness.

1) *5-Ketomilbemycin D* (**4**). To a solution of milbemycin D (**1**, 2.0 g) in acetone (50 ml) was added active MnO_2 (15.6 g). The mixture was vigorously stirred for 2 hr at room temperature, and the reaction mixture was filtered through Celite®. The filtrate was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography with hexane–ethyl acetate (5 : 1) to afford **4** (1.59 g, 80%). IR ν_{max} (KBr) cm^{-1}: 3470, 2961, 2928, 2870, 1739, 1715, 1685, 1641, 1457, 1435, 1384, 1368, 1336, 1315, 1275, 1244, 1181, 1119, 1046, 1009, 887. ^{1}H-NMR ($CDCl_3$) δ: 6.58–6.57 (1H, m, H-C(3)); 5.87 (1H, dt, J_d = 11.3 Hz, J_t = 2.4 Hz, H-C(9)); 5.74 (1H, dd, J = 14.5 and 11.3 Hz, H-C(10)); 5.49–5.35 (2H, m, H-C(11), H-C(19)); 4.99–4.93 (1H, m, H-C(15)); 4.75 (1H, dd, J = 14.5 and 2.4 Hz, CH-C(8)); 4.74 (1H, dd, J = 14.5 and 2.4 Hz, CH-C(8)); 4.02 (1H, s, HO-C(7)); 3.86 (1H, s, H-C(6)); 3.65–3.55 (2H, m, H-C(2), H-C(17)); 3.09 (1H, dd, J = 9.7 and 2.0 Hz, H-C(25)); 2.51–2.34 (1H, m, H-C(12)); 2.26–2.15 (3H, m, H-C(13), H_2-C(16)); 2.05–1.99 (1H, m, H-C(20)); 1.95–1.79 (3H, m, H-C(13), H-C(18), CH-C(25)); 1.90–1.89 (3H, m, CH_3-C(4)); 1.69–1.41 (5H, m, H_2-C(22), H_2-C(23), H-C(24)); 1.53 (3H, s, CH_3-C(14)); 1.37 (1H, t, J = 11.7 Hz, H-C(20)); 1.06 (3H, d, J = 6.9 Hz, β-CH_3-C(25)); 1.01 (3H, d, J = 6.9 Hz, CH_3-C(12)); 0.95–0.80 (1H, m, H-C(18)); 0.87 (3H, d, J = 6.9 Hz, β-CH_3-C(25)); 0.81 (3H, d, J = 6.0 Hz, CH_3-C(24)). MS *m/z*: 554 (M^+, $C_{33}H_{46}O_7$), 536, 278, 259, 209, 181, 151. HR-MS *m/z*: calcd. for $C_{33}H_{46}O_7$–H_2O, 536.3138; found, 536.3130.

2) *5-Ketomilbemycin A_4* (**5**) *and A_3* (**6**). By a MnO_2 oxidation similar to that just described, milbemycin A_4 (**2**) and A_3 (**3**) were converted to **5** and **6**, respectively (70% and 78%).

5. IR ν_{max} (KBr) cm^{-1}: 3464, 2961, 2928, 2873, 1737, 1715, 1684, 1641, 1455, 1436, 1377, 1336, 1315, 1274, 1244, 1181, 1102, 1031, 989, 887. ^{1}H-NMR ($CDCl_3$) δ: 6.55–6.54 (1H, m, H-C(3)); 5.87 (1H, dt, J_d = 11.3 Hz, J_t = 2.4 Hz, H-C(9)); 5.74 (1H, dd, J = 14.7 and 11.3 Hz, H-C(10)); 5.48–5.41 (2H, m, H-C(11), H-C(19)); 4.99–4.95 (1H, m, H-C(15)); 4.75 (1H, dd, J = 14.5 and 2.4 Hz, CH-C(8)); 4.73 (1H, dd, J = 14.5 and 2.4 Hz, CH-C(8)); 4.03 (1H, s, HO-C(7)); 3.85 (1H, s, H-C(6)); 3.62–3.55 (2H, m, H-C(2), H-C(17)); 3.08 (1H, td, J_t = 9.8 Hz, J_d = 2.9 Hz, H-C(25)); 2.50–2.37 (1H, m, H-C(12)); 2.26–2.16 (3H, m, H-C(13), H_2-C(16)); 2.05–2.01 (1H, m, H-C(20)); 1.91–1.80 (2H, m, H-C(13), H-C(18)); 1.90–1.89 (3H, m, CH_3-C(4));

1.76–1.24 (8H, m, H-C(20), H_2-C(22), H_2-C(23), H-C(24), CH_2-C(25)); 1.53 (3H, s, CH_3-C(14)); 1.02–0.99 (6H, m, CH_3-C(12), β-CH_3-C(25)); 0.95–0.80 (1H, m, H-C(18)); 0.83 (3H, d, J=6.8 Hz, CH_3-C(24)). MS *m/z*: 540 (M^+, $C_{32}H_{44}O_7$), 522, 414, 264, 195, 167, 151. HR-MS *m/z*: calcd. for $C_{32}H_{44}O_7$, 540.3087; found, 540.3073.

6. IR ν_{max} (KBr) cm^{-1}: 3471, 2966, 2925, 2874, 1735, 1715, 1679, 1639, 1450, 1380, 1335, 1315, 1275, 1244, 1181, 1116, 1095, 1056, 1036, 996, 888. ^{1}H-NMR ($CDCl_3$) δ: 6.54–6.53 (1H, m, H-C(3)); 5.86 (1H, dt, J_d=11.2 Hz, J_t=2.4 Hz, H-C(9)); 5.74 (1H, dd, J=14.6 and 11.2 Hz, H-C(10)); 5.49–5.39 (2H, m, H-C(11), H-C(19)); 5.02–4.98 (1H, m, H-C(15)); 4.75 (1H, dd, J=14.5 and 2.4 Hz, CH-C(8)); 4.73 (1H, dd, J=14.5 and 2.4 Hz, CH-C(8)); 4.04 (1H, s, HO-C(7)); 3.85 (1H, s, H-C(6)); 3.59–3.52 (2H, m, H-C(2), H-C(17)); 3.32–3.24 (1H, m, H-C(25)); 2.50–2.36 (1H, m, H-C(12)); 2.26–2.20 (3H, m, H-C(13), H_2-C(16)); 2.04–1.99 (1H, m, H-C(20)); 1.93–1.80 (2H, m, H-C(13), H-C(18)); 1.90–1.89 (3H, m, CH_3-C(4)); 1.69–1.48 (4H, m, H_2-C(22), H_2-C(23)); 1.53 (3H, s, CH_3-C(14)); 1.37 (1H, t, J=11.7 Hz, H-C(20)); 1.34–1.20 (1H, m, H-C(24)); 1.16 (3H, d, J=6.4 Hz, CH_3-C(25)); 1.01 (3H, d, J=6.8 Hz, CH_3-C(12)); 0.92–0.83 (1H, m, H-C(18)); 0.84 (3H, d, J=6.8 Hz, CH_3-C(24)): MS *m/z*: 526 (M^+, $C_{31}H_{42}O_7$), 508, 400, 250, 181, 153, 151. HR-MS *m/z*: calcd. for $C_{31}H_{42}O_7$, 526.2930; found, 526.2906.

3) *5-Ketomilbemycin D 5-oxime* (**7**; *general procedure A*). A solution of NH_2OH–HCl (125 mg) in water (2 ml) was added dropwise to a solution containing **4** (166 mg) in methanol (2 ml)–dioxane (2 ml). The mixture was stirred at room temperature for 6 hr and then condensed under reduced pressure. The residue was dissolved in ether, washed with water, dried (Na_2SO_4), and concentrated *in vacuo*. After purification by silica gel column chromatography (3:1 hexane–ethyl acetate), 145 mg of **7** was obtained (85.0%). IR ν_{max} (KBr) cm^{-1}: 3456, 2962, 2929, 2872, 1714, 1675, 1635, 1457, 1434, 1384, 1367, 1337, 1273, 1246, 1180, 1119, 1040, 1009, 1000, 985, 963, 867. ^{1}H-NMR ($CDCl_3$) δ: 5.87 (1H, dt, J_d=11.3 Hz, J_t=2.0 Hz, H-C(9)); 5.83–5.82 (1H, m, H-C(3)); 5.75 (1H, dd, J=14.5 and 11.3 Hz, H-C(10)); 5.45–5.36 (2H, m, H-C(11), H-C(19)); 4.98–4.93 (1H, m, H-C(15)); 4.76 (1H, dd, J=14.5 and 2.0 Hz, CH-C(8)); 4.73–4.66 (1H, m, CH-C(8)); 4.68 (1H, s, H-C(6)); 3.65–3.54 (1H, m, H-C(17)); 3.40–3.38 (1H, m, H-C(2)); 3.08 (1H, br.d, J=9.3 Hz, H-C(25)); 2.50–2.34 (1H, m, H-C(12)); 2.26–2.14 (3H, m, H-C(13), H_2-C(16)); 2.03–1.97 (1H, m, H-C(20)); 1.94–1.93 (3H, m, CH_3-C(4)); 1.94–1.80 (3H, m, H-C(13), H-C(18), CH-C(25)); 1.68–1.40 (5H, m, H_2-C(22), H_2-C(23), H-C(24)); 1.53 (3H, s, CH_3-C(14)); 1.36 (1H, t, J=11.7 Hz, H-C(20)); 1.05 (3H, d, J=7.3 Hz, β-CH_3-C(25)); 1.01 (3H, d, J=6.8 Hz, CH_3-C(12)); 0.94–0.80 (1H, m, H-C(18)); 0.87 (3H, d, J=7.3 Hz, β-CH_3-C(25)); 0.81 (3H, d, J=5.6 Hz, CH_3-C(24)). MS *m/z*: 569 (M^+, $C_{33}H_{47}O_7N$), 551, 535, 497, 455, 292, 274, 259, 209, 181, 151. HR-MS *m/z*: calcd. for $C_{33}H_{47}O_7N$–H_2O, 551.3247; found, 551.3245.

4) *5-Ketomilbemycin A_4 5-oxime* (**8**) *and 5-ketomilbemycin A_3 5-oxime* (**9**). Compounds **8** and **9** were synthesized according to general procedure A (91.4% and 90.4%).

8. IR ν_{max} (KBr) cm^{-1}: 3446, 2960, 2927, 2874, 1714, 1674, 1632, 1456, 1435, 1375, 1337, 1272, 1246, 1180, 1168, 1115, 1103, 1057, 1031, 989, 963, 866. ^{1}H-NMR ($CDCl_3$) δ: 5.87 (1H, dt, J_d=11.3 Hz, J_t=2.0 Hz, H-C(9)); 5.80 (1H, s, H-C(3)); 5.75 (1H, dd, J=14.4 and 11.3 Hz, H-C(10)); 5.48–5.35 (2H, m, H-C(11), H-C(19)); 4.99–4.93 (1H, m, H-C(15)); 4.76 (1H, dd, J=14.5 and 2.0 Hz, CH-C(8)); 4.73–4.66 (1H, m, CH-C(8)); 4.67 (1H, s, H-C(6)); 3.62–3.52 (1H, m, H-C(17)); 3.40–3.38 (1H, m, H-C(2)); 3.08 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-C(25)); 2.51–2.35 (1H, m, H-C(12)); 2.26–2.17 (3H, m, H-C(13), H_2-C(16)); 2.04–1.97 (1H, m, H-C(20)); 1.93 (3H, s, CH_3-C(4)); 1.93–1.80 (2H, m, H-C(13), H-C(18)); 1.76–1.25 (8H, m, H-C(20), H_2-C(22), H_2-C(23), H-C(24), CH_2-C(25)); 1.53 (3H, s, CH_3-C(14)); 1.02–0.97 (6H, m, CH_3-C(12), β-CH_3-C(25)); 0.95–0.80 (1H, m, H-C(18)); 0.83 (3H, d, J=6.5 Hz, CH_3-C(24)). MS *m/z*: 555 (M^+, $C_{32}H_{45}O_7N$), 537, 520, 292, 274, 245, 195, 151. HR-MS *m/z*: calcd. for $C_{32}H_{45}O_7N$, 555.3196; found, 555.3188.

9. IR ν_{max} (KBr) cm^{-1}: 3436, 2966, 2927, 2876, 1713, 1674, 1638, 1451, 1378, 1337, 1274, 1246, 1181, 1169, 1116, 1096, 1084, 1056, 1036, 994, 964, 853. ^{1}H-NMR ($CDCl_3$) δ: 8.13 (1H, br.s, HO–N=C(5)); 5.87 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-C(9)); 5.80–5.71 (2H, m, H-C(3), H-C(10)); 5.51–5.33 (2H, m, H-C(11), H-C(19)); 5.01–4.96 (1H, m, H-C(15)); 4.76 (1H, dd, J=14.5 and 2.4 Hz, CH-C(8)); 4.72–4.67 (1H, m, CH-C(8)); 4.67 (1H, s, H-C(6)); 4.11 (1H, br.s, HO-C(7)); 3.60–3.51 (1H, m, H-C(17)); 3.39–3.37 (1H, m, H-C(2)); 3.32–3.22 (1H, m, H-C(25)); 2.52–2.36 (1H, m, H-C(12)); 2.26–2.20 (3H, m, H-C(13), H_2-C(16)); 2.04–1.80 (3H, m, H-C(13), H-C(18), H-C(20)); 1.94–1.93 (3H, m, CH_3-C(4)); 1.69–1.48 (4H, m, H_2-C(22), H_2-C(23)); 1.53 (3H, s, CH_3-C(14)); 1.37 (1H, t, J=11.7 Hz, H-C(20)); 1.34–1.20 (1H, m, H-C(24)); 1.15 (3H, d, J=6.0 Hz, CH_3-C(25)); 1.01 (3H, d, J=6.8 Hz, CH_3-C(12)); 0.95–0.80 (1H, m, H-C(18)); 0.84 (3H, d, J=6.4 Hz, CH_3-C(24)). MS *m/z*: 541 (M^+, $C_{31}H_{43}O_7N$), 541, 523, 507, 292, 274, 231, 181, 153. HR-MS *m/z*: calcd. for $C_{31}H_{43}O_7N$, 541.3040; found, 541.3057.

5) *Oximation of 5-ketomilbemycin D* (**4**) *with free hydroxylamine*. To a solution of hydroxylamine hydrochloride (209 mg) and sodium acetate (245 mg) in methanol (10 ml) was added compound (**4**, 554 mg) in an ice bath. After stirring for 2 hr at 0°C, the mixture was concentrated *in vacuo* and diluted with ethyl acetate (100 ml) and benzene (100 ml). The resulting mixture was washed with H_2O and brine, dried over Na_2SO_4, and evaporated under reduced pressure. Silica gel column chromatography of the residue (3:2 hexane–ethyl acetate) gave 130 mg of **7** (22.8%) and 210 mg of **10** (34.9%). **10**. IR ν_{max} (KBr) cm^{-1}: 3381, 2962, 2930, 2873, 1723, 1674, 1640, 1457, 1434, 1384, 1376, 1334, 1273, 1246, 1185, 1165, 1119, 1040, 1009, 980, 964, 814.

^{1}H-NMR ($CDCl_3$) δ: 5.88 (1H, br. d, J=11.3 Hz, H-C(9)); 5.73 (1H, dd, J=14.5 and 11.3 Hz, H-C(10)); 5.39 (1H, dd, J=14.5, and 10.1 Hz, H-C(11)); 5.31–5.18 (1H, m, H-C(19)); 4.98–4.92 (1H, m, H-C(15)); 4.61 (1H, s, CH-C(8)); 4.60 (1H, s, CH-C(8)); 4.16–4.04 (1H, m, H-C(4)); 3.99 (1H, s, H-C(6)); 3.63–3.53 (1H, m, H-C(17)); 3.46–3.43 (1H, m, H-C(3)); 3.09–3.05 (2H, m, H-C(2), H-C(25)); 2.50–2.35 (1H, m, H-C(12)); 2.25–2.15 (3H, m, H-C(13), H_2-C(16)); 2.12–2.04 (1H, m, H-C(20)); 1.93–1.83 (3H, m, H-C(13), H-C(18), CH-C(25)); 1.67–1.40 (5H, m, H_2-C(22), H_2-C(23), H-C(24)); 1.54 (3H, s, CH_3-C(14)); 1.36–1.23 (1H, m, H-C(20)); 1.26 (3H, d, J=7.3 Hz, CH_3-C(4)); 1.04 (3H, d, J=6.9 Hz, β-CH_3-C(25)); 1.00 (3H, d, J=6.4 Hz, CH_3-C(12)); 0.93–0.80 (1H, m, H-C(18)); 0.87 (3H, d, J=6.9 Hz, β-CH_3-C(25)); 0.80 (3H, d, J=5.6 Hz, CH_3-C(24)); MS m/z: 602 (M^+, $C_{33}H_{50}O_8N_2$), 586, 570, 256, 209, 181, 149. HR-MS m/z: calcd. for $C_{33}H_{50}O_8N_2$, 602.3568; found, 602. 3558.

6) *5-Ketomilbemycin D 5-O-acetyloxime* (**11**; *general procedure B*). To a solution of **7** (64.0 mg) in acetonitrile (1 ml) in an ice bath was added DABCO (1,4-diazabicyclo[2.2.2]octane, 15.1 mg) and acetyl chloride (10 μl). After 30 min, the reaction mixture was poured into ice-cold water and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by preparative TLC (3 : 1 hexane–ethyl acetate) to give 63.2 mg of **11** (92.4%). IR ν_{max} (KBr) cm^{-1}: 3466, 2962, 2929, 2872, 1781, 1740, 1714, 1674, 1639, 1456, 1434, 1384, 1366, 1336, 1316, 1272, 1190, 1183, 1119, 1047, 1009, 1000, 963, 944. ^{1}H-NMR ($CDCl_3$) δ: 6.03–6.02 (1H, m, H-C(3)); 5.88 (1H, dt, J_d=11.3 Hz, J_t=2.0 Hz, H-C(9)); 5.75 (1H, dd, J=14.0 and 11.3 Hz, H-C(10)); 5.47–5.35 (2H, m, H-C(11), H-C(19)); 5.00–4.91 (1H, m, H-C(15)); 4.75 (1H, dd, J=14.5 and 2.0 Hz, CH-C(8)); 4.71 (1H, dd, J=14.5 and 2.0 Hz, CH-C(8)); 4.60 (1H, s, H-C(6)); 3.96 (1H, s, HO-C(7)); 3.66–3.53 (1H, m, H-C(17)); 3.42–3.39 (1H, m, H-C(2)); 3.08 (1H, dd, J=9.6 and 2.0 Hz, H-C(25)); 2.52–2.35 (1H, m, H-C(12)); 2.24 (3H, s, $COCH_3$); 2.26–2.13 (3H, m, H-C(13), H_2-C(16)); 2.06–2.05 (3H, m, CH_3-C(4)); 2.05–1.97 (1H, m, H-C(20)); 1.93–1.78 (3H, m, H-C(13), H-C(18), CH-C(25)); 1.67–1.40 (5H, m, H_2-C(22), H_2-C(23), H-C(24)); 1.53 (3H, s, CH_3-C(14)); 1.36 (1H, t, J=11.6 Hz, H-C(20)); 1.05 (3H, d, J=7.0 Hz, β-CH_3-C(25)); 1.01 (3H, d, J=7.0 Hz, CH_3-C(12)); 0.93–0.79 (1H, m, H-C(18)); 0.87 (3H, d, J=7.0 Hz, β-CH_3-C(25)); 0.80 (3H, d, J=5.8 Hz, CH_3-C(24)). MS m/z: 611 (M^+, $C_{35}H_{49}O_8N$), 569, 551, 274, 209, 181, 151. HR-MS m/z: calcd. for $C_{35}H_{49}O_8N$, 611.3458; found, 611.3471.

7) *5-Ketomilbemycin A_4 5-O-acetyloxime* (**12**). This compound was prepared from **8** (57.1 mg) in an 85% yield by the procedure described for **11**. IR ν_{max} (KBr) cm^{-1}: 3465, 2960, 2927, 2872, 1780, 1740, 1714, 1674, 1638, 1455, 1436, 1367, 1336, 1317, 1272, 1243, 1190, 1182, 1115, 1103, 1080, 1045, 1030, 990, 964, 944, 907. ^{1}H-NMR ($CDCl_3$) δ: 6.01–6.00 (1H, m, H-C(3)); 5.88 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-C(9)); 5.75 (1H, dd, J=14.1 and 11.3 Hz, H-C(10)); 5.49–5.38 (2H, m, H-C(11), H-C(19)); 4.99–4.94 (1H, m, H-C(15)); 4.75 (1H, dd, J=14.1 and 2.4 Hz, CH-C(8)); 4.70 (1H, dd, J=14.1 and 2.4 Hz, CH-C(8)); 4.60 (1H, s, H-C(6)); 3.98 (1H, br. s, HO-C(7)); 3.62–3.52 (1H, m, H-C(17)); 3.41–3.38 (1H, m, H-C(2)); 3.08 (1H, td, J_t=9.7 Hz, J_d=2.4 Hz, H-C(25)); 2.51–2.36 (1H, m, H-C(12)); 2.26–2.17 (3H, m, H-C(13), H_2-C(16)); 2.24 (3H, s, $COCH_3$); 2.06–2.04 (3H, m, CH_3-C(4)); 2.05–1.98 (1H, m, H-C(20)); 1.92–1.78 (2H, m, H-C(13), H-C(18)); 1.74–1.23 (8H, m, H-C(20), H_2-C(22), H_2-C(23), H-C(24), CH_2-C(25)); 1.54 (3H, s, CH_3-C(14)); 1.02–0.97 (6H, m, CH_3-C(12), β-CH_3-C(25)); 0.95–0.80 (1H, m, H-C(18)); 0.83 (3H, d, J=6.4 Hz, CH_3-C(24)). MS m/z: 597 (M^+, $C_{34}H_{47}O_8N$), 539, 395, 274, 195, 167, 151. HR-MS m/z: calcd. for $C_{34}H_{47}O_8N$, 597.3302; found, 597.3311.

8) *5-Ketomilbemycin D 5-O-ethoxycarbonyloxime* (**13**). By an analogous method to that used for the preparation of **11**, compound **7** (63.4 mg) in acetonitrile (1 ml) was treated with ethyl chloroformate (13 μl) in the presence of DABCO (15 mg), and purified by preparative TLC (3 : 1 hexane–ethyl acetate) to give **13** (68.4 mg, 96.4%). IR ν_{max} (KBr) cm^{-1}: 3468, 2962, 2929, 2871, 1785, 1740, 1714, 1674, 1636, 1457, 1435, 1383, 1369, 1335, 1317, 1272, 1224, 1180, 1119, 1068, 1046, 1008, 998, 965, 942, 862. ^{1}H-NMR ($CDCl_3$) δ: 6.03–6.02 (1H, m, H-C(3)); 5.87 (1H, dt, J_d=11.1 Hz, J_t=2.3 Hz, H-C(9)); 5.74 (1H, dd, J=14.0 and 11.1 Hz, H-C(10)); 5.46–5.33 (2H, m, H-C(11), H-C(19)); 4.99–4.92 (1H, m, H-C(15)); 4.78 (1H, dd, J=14.5 and 2.3 Hz, CH-C(8)); 4.70 (1H, dd, J=14.5 and 2.3 Hz, CH-C(8)); 4.61 (1H, s, H-C(6)); 4.37–4.28 (2H, m, CH_2OCO-N=C(5)); 3.92 (1H, br. s, HO-C(7)); 3.65–3.55 (1H, m, H-C(17)); 3.42–3.38 (1H, m, H-C(2)); 3.08 (1H, dd, J=9.3 and 2.0 Hz, H-C(25)); 2.52–2.35 (1H, m, H-C(12)); 2.26–2.16 (3H, m, H-C(13), H_2-C(16)); 2.06 (3H, s, CH_3-C(4)); 2.04–1.97 (1H, m, H-C(20)); 1.92–1.77 (3H, m, H-C(13), H-C(18), CH-C(25)); 1.70–1.31 (6H, m, H-C(20), H_2-C(22), H_2-C(23), H-C(24)); 1.53 (3H, s, CH_3-C(14)); 1.36 (3H, t, J=7.0 Hz, CH_3CH_2OCO-N=C(5)); 1.05 (3H, d, J=7.0 Hz, β-CH_3-C(25)); 1.01 (3H, d, J=7.0 Hz, CH_3-C(12)); 0.93–0.80 (1H, m, H-C(18)); 0.87 (3H, d, J=7.0 Hz, β-CH_3-C(25)); 0.80 (3H, d, J=5.8 Hz, CH_3-C(24)). MS m/z: 641 (M^+, $C_{36}H_{51}O_9N$), 598, 569, 551, 274, 209, 181, 151. HR-MS m/z: calcd. for $C_{36}H_{51}O_9N$, 641.3564; found, 641.3564.

9) *5-Ketomilbemycin D 5-O-methylcarbamoyloxime* (**14**). Triethylamine (40 μl) and methylisocyanate (20 μl) were added to a solution of **7** (70.4 mg) in CH_2Cl_2 (2 ml). The solution was stirred at room temperature for 6 hr, and condensed *in vacuo*. The residue was purified by preparative TLC (1 : 1 hexane–ethyl acetate), affording 60.7 mg of **14** (78.2%). IR ν_{max} (KBr) cm^{-1}: 3439, 3385, 2961, 2929, 2873, 1732, 1674, 1636, 1507, 1457, 1384, 1367, 1336, 1317, 1274,

1243, 1215, 1181, 1118, 1089, 1069, 1044, 1009, 998, 964, 950, 908, 870. ^{1}H-NMR ($CDCl_3$) δ: 6.22–6.17 (1H, m, NH); 6.05–6.03 (1H, m, H-C(3)); 5.85 (1H, dt, $J_d = 11.3$ Hz, $J_t = 2.4$ Hz, H-C(9)); 5.74 (1H, dd, $J = 14.5$ and 11.3 Hz, H-C(10)); 5.46–5.33 (2H, m, H-C(11), H-C(19)); 4.98–4.93 (1H, m, H-C(15)); 4.76 (1H, dd, $J = 14.5$ and 2.4 Hz, CH-C(8)); 4.68 (1H, dd, $J = 14.5$ and 2.4 Hz, CH-C(8)); 4.67 (1H, s, H-C(6)); 3.91 (1H, s, HO-C(7)); 3.65–3.55 (1H, m, H-C(17)); 3.41–3.39 (1H, m, H-C(2)); 3.08 (1H, br, d, $J = 9.3$ Hz, H-C(25)); 2.93 (3H, d, $J = 4.8$ Hz, NCH_3); 2.52–2.35 (1H, m, H-C(12)); 2.26–2.14 (3H, m, H-C(13), H_2-C(16)); 2.04–1.77 (4H, m, H-C(13), H-C(18), H-C(20), CH-C(25)); 2.00–1.99 (3H, m, CH_3-C(4)); 1.68–1.40 (5H, m, H_2-C(22), H_2-C(23), H-C(24)); 1.53 (3H, s, CH_3-C(14)); 1.36 (1H, t, $J = 11.7$ Hz, H-C(20)); 1.05 (3H, d, $J = 6.9$ Hz, β-CH_3-C(25)); 1.02 (3H, d, $J = 6.8$ Hz, CH_3-C(12)); 0.93–0.80 (1H, m, H-C(18)); 0.87 (3H, d, $J = 6.9$ Hz, β-CH_3-C(25)); 0.81 (3H, d, $J = 5.6$ Hz, CH_3-C(24)). MS *m/z*: 569 ($M - CH_3NCO$, $C_{33}H_{47}O_7N$), 551, 535, 497, 292, 274, 259, 209, 181, 151, 57. HR-MS *m/z*: calcd. for $C_{33}H_{47}O_7N$, 569.3352; found, 569.3352.

10) *5-Ketomilbemycin A_4 5-O-dimethylcarbamoyloxime* (**15**). By using $ClCONMe_2$ (16.0 μl), DABCO (15.0 mg) and acetonitrile (1 ml), **8**, (61.8 mg) was converted to 63.8 mg of **15** (91.9%) by an analogous method to that described for **11**. IR ν_{max} (KBr) cm^{-1}: 3470, 2958, 2928, 2873, 1744, 1675, 1639, 1488, 1453, 1381, 1336, 1316, 1272, 1245, 1165, 1115, 1103, 1046, 1016, 990, 964, 944, 907, 861, 821, 754. ^{1}H-NMR ($CDCl_3$) δ: 5.94–5.86 (2H, m, H-C(3), H-C(9)); 5.74 (1H, dd, $J = 14.5$ and 11.3 Hz, H-C(10)); 5.49–5.38 (2H, m, H-C(11), H-C(19)); 4.99–4.94 (1H, m, H-C(15)); 4.71 (2H, d, $J = 2.0$ Hz, CH_2-C(8)); 4.57 (1H, s, H-C(6)); 4.00 (1H, s, HO-C(7)); 3.63–3.53 (1H, m, H-C(17)); 3.41–3.38 (1H, m, H-C(2)); 3.08 (1H, td, $J_t = 9.7$ Hz, $J_d = 2.4$ Hz, H-C(25)); 2.99 (6H, s, $N(CH_3)_2$); 2.51–2.35 (1H, m, H-C(12)); 2.26–2.17 (3H, m, H-C(13), H_2-C(16)); 2.08–2.07 (3H, m, CH_3-C(4)); 2.05–1.98 (1H, m, H-C(20)); 1.92–1.78 (2H, m, H-C(13), H-C(18)); 1.74–1.23 (8H, m, H-C(20), H_2-C(22), H_2-C(23), H-C(24), CH_2-C(25)); 1.54 (3H, s, CH_3-C(14)); 1.02–0.97 (6H, m, CH_3-C(12), β-CH_3-C(25)); 0.95–0.80 (1H, m, H-C(18)); 0.83 (3H, d, $J = 6.9$ Hz, CH_3-C(24)). MS *m/z*: 626 (M^+, $C_{35}H_{50}O_8N_2$), 608, 539, 537, 395, 274, 195, 167, 151. HR-MS *m/z*: calcd. for $C_{35}H_{50}O_8N_2 - H_2O$, 608.3461; found, 608.3459.

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