SYNTHESIS AND ANTHELMINTIC ACTIVITY OF 13-ALKOXYMILBEMYCIN DERIVATIVES

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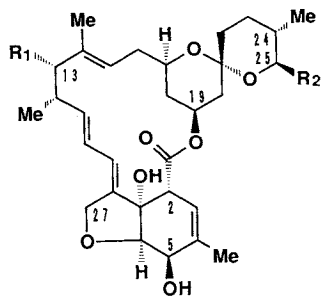
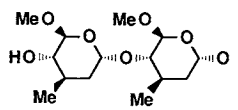
The synthesis of milbemycin derivatives having alkyloxy groups at the C-13 position was studied and a series of these analogs of milbemycin A_4 and A_3 was prepared by the reaction of 13-iodomilbemycin with a variety of alcohols. 13β-Phenethyloxy derivatives were found to possess excellent anthelmintic activity. Especially, the activities of derivatives with *N*-substituted 4-aminophenethyloxy groups were comparable to or superior to ivermectin against *Nippostrongylus brasiliensis* in rats.

The milbemycins are a family of 16-membered ring macrocyclic lactones isolated from *Streptomyces hydroscopicus*[1], which are known to possess high anthelmintic, acaricidal, and insecticidal activity[2]. Both the native compound, which consists of a 3:7 mixture of milbemycin A_3 (**1a**) and A_4 (**1b**), and its 5-oxime[3] are now in commercial use as agricultural chemicals and for the market of small animal health, respectively. The avermectins, which have closely related structures and biological activities to the milbemycins, are produced by a culture of *Streptomyces avermitilis*[4]. Ivermectin (**1d**), a mixture of 22,23-dihydroavermectin B_{1a} and B_{1b}, is widely used as a potent antiparasitic agent for controlling parasitic infections of livestock[5]. The fundamental structural difference between the milbemycins and ivermectin is that the former lack the α-L-oleandosyl-α-L-oleandosyl group at the C-13 position. Merck chemists reported that the C-13 substituent of ivermectin affects the insecticidal activities markedly[6], and a large number of milbemycin derivatives, which have a wide variety of substituents at C-13 position such as halogen[7,8], acyloxy[9,10], alkyl[11,12], glycosydic residue[13] and others[14~17], have been prepared with the aim of obtaining a highly potent drug.

While 13-alkoxymilbemycins (**1**, R_1 = RO) are of special interest due to the structural resemblance to avermectin, only a few examples of such compounds have been reported, presumably because no

Fig. 1.



	R_1	R_2
Milbemycin A_3 (**1a**)	H	Me
Milbemycin A_4 (**1b**)	H	Et
Milbemycin D (**1c**)	H	*i*-Pr
Ivermectin (**1d**)		*s*-Bu + *i*-Pr

practical method was available for the synthesis of these compounds. This paper deals with the synthesis and anthelmintic activities of 13-alkoxymilbemycins.

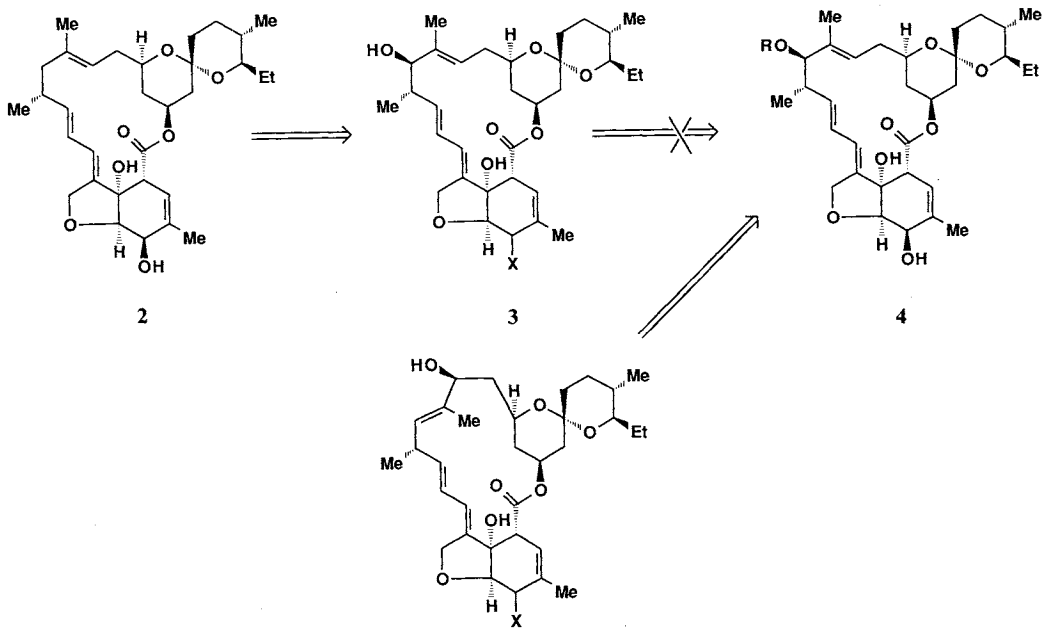
Chemistry

Alkylations of 13-hydroxymilbemycins (**3**) seem to be a most feasible route for the syntheses of **4**. Merck workers reported the preparation of some 13α-alkoxymethoxy derivatives by the reaction of 22,23-dihydroavermectin aglycon (**1**, R_1=OH, R_2=*s*-Bu), which has an α-hydroxy residue at C-13, with corresponding alkoxymethyl chlorides[6]. However, no examples of the synthesis of 13-alkoxymilbemycins (**4**) by the reaction of 13-hydroxy derivative (**3**) with the usual alkylating reagents such as alkyl iodide have been reported[6], presumably because the hydroxy group at the C-13 position is quite unreactive toward the usual alkylating reagents because of steric hindrance. Some simple 13-alkoxy derivatives have been prepared by another method which consists of the solvolysis of appropriate intermediates. Thus, 13β-methoxy and 13β-ethoxy milbemycin (**4**, R=Me or Et) were prepared by acid catalyzed solvolysis of 15-hydroxymilbemycin (**5**) in methanol or ethanol[18], and, the 13α-methoxy derivative (**1**, R=MeO) was obtained by methanolysis of the 13-(2-nitrobenzenesulfonyl)oxy derivative of the avermectin analog[6]. Therefore, there was a need for general and practical method for the preparation of 13-alkoxymilbemycin (**4**) in order to study the anthelmintic activity of these compounds.

We tried several methods for the preparation of 13-alkyloxymilbemycins (**4**) by using 13-hydroxy-5-oxomilbemycin A_4 (**6**) as a starting material. The compound is readily available[19] and $NaBH_4$ reduction of the 5-oxo group can selectively regenerate the 5β-hydroxy moiety, which is essential for potent anthelmintic activity.

The reaction of **6** with methyl iodide in the presence of Ag_2O, which had been used for the methylation

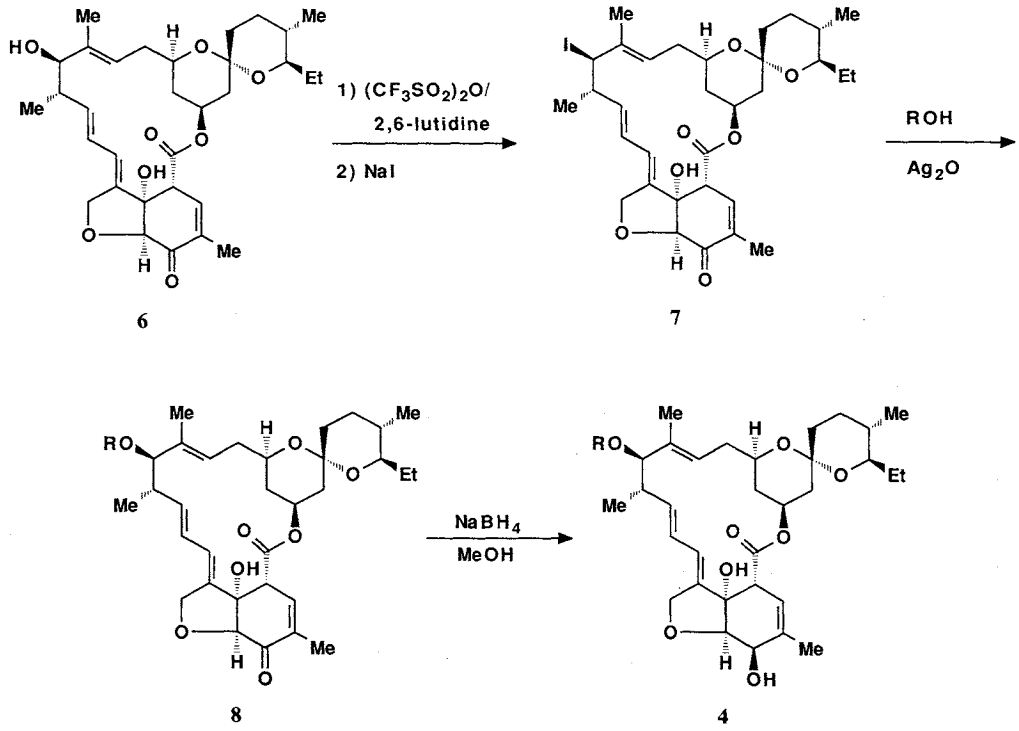
Scheme 1.



2 3 4

5

Scheme 2.



6 7 8 4

of the 5-hydroxy group of milbemycins, gave no alkylated product. More vigorous treatment resulted in decomposition of the starting material (**6**). We then, therefore, tried the reaction of 13-iodomilbemycin (**7**) with alcohols. Treatment of the 13-hydroxymilbemycin (**6**) with trifluoromethanesulfonic anhydride and 2,6-lutidine followed by trapping of the resulting triflate with sodium iodide gave the 13β-iodide (**7**) as a crystalline product, although the yield was very poor (12%). Reaction of **7** with benzyl alcohol in the presence of Ag_2O in THF proceeded smoothly to afford the desired 13β-benzyloxy derivative (**8**, R = benzyl) in 55% yield.

The β-configuration at the 13-position of these compounds (**7** and **8**) was confirmed by their ^{1}H NMR spectra with a characteristic doublet ($J_{12,13}=9\sim11$ Hz) of the C_{13}-H[6,20,21]. Exclusive formation of the β-substituted products in these reactions indicate that substitutions at the C-13 position obviously take place in the β-site *via* C_{13}-C_{15} allylic cation intermediate in which the α-site is sterically crowded[6]. This finding shows that a variety of 13-alkoxymilbemycins can be prepared *via* the 13-iodomilbemycin (**7**) but the total yield is very low. Therefore, we tried to optimize this procedure.

The iodide (**7**) could be prepared by using the novel method described below: Reaction of the 13-hydroxymilbemycin (**6**) with 2-chloroformyl-1,2,4-triazolo[4,3-*a*]pyridin-3-one (**9**)[22] gave the urethane intermediate (**10**), which contains an active-amide bond, in nearly quantitative yield. Treatment of **10** with zinc iodide gave the 13β-iodide (**7**) in high yield. This reaction is probably initiated by coordination of the zinc ion to the carbonyl group of the triazolopyridine ring which causes cleavage of the active-amide bond to give the C_{13}-C_{15} allylic cation with emission of carbon dioxide.

This procedure seems to be very useful for the conversion of allyl alcohols into allyl iodides, especially

Scheme 3.

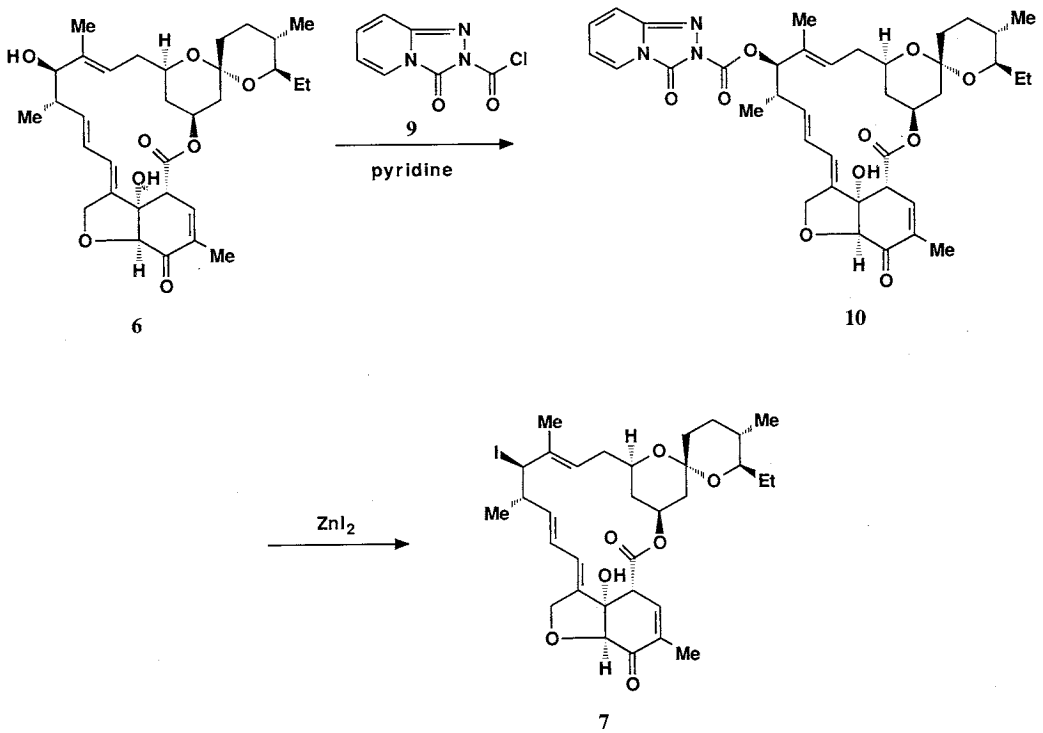


Table 1. Reaction conditions and products of the etherification.

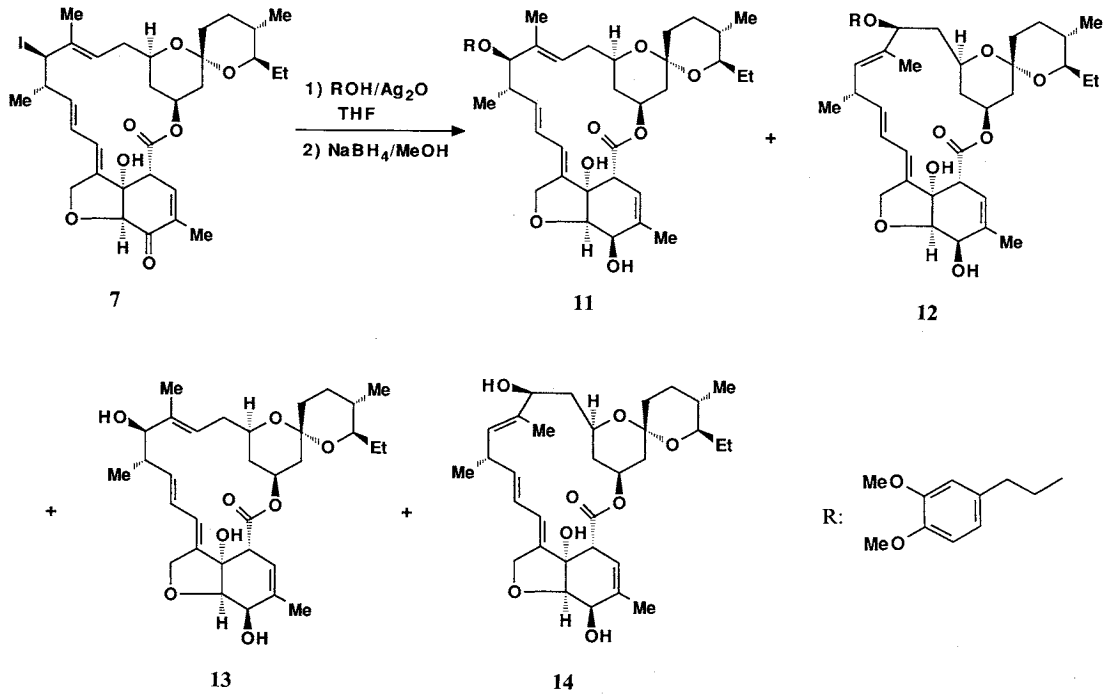
Additive	Solvent	Reaction condition	Yield (%)			
			11	**12**	**13**	**14**
Ag_2O	THF	rt[b], 30 minutes	36.8	19.5	28.2	9.0
CF_3SO_3Ag	THF	rt, 20 minutes	50.4	29.4	7.9	4.1
HgI_2	THF	rt, 30 minutes	63.1	21.7	10.4	6.9
HgI_2	DCE[a]	rt, 90 minutes	75.8	3.3	9.0	3.4

[a] 1,2-dichloroethane.
[b] room temperature.

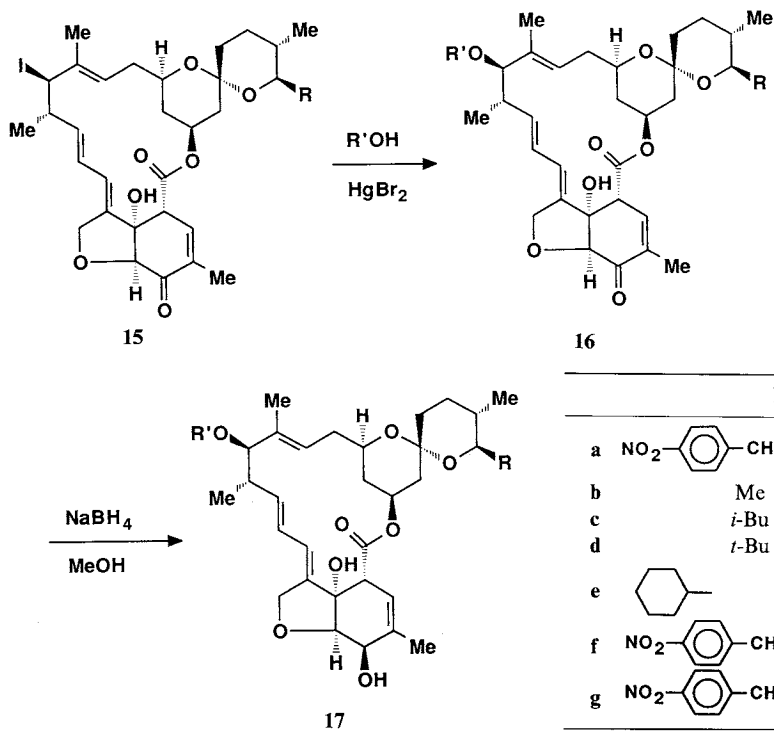
in the synthesis of complex molecules, since the conversion proceeds under very mild conditions in good yields.

Another problem in the synthesis of 13-alkoxymilbemycins was an inadequate yield in the etherification step. Reaction of the 13-iodide (**7**) with 3,4-dimethoxyphenethyl alcohol was examined under various conditions as shown in Table 1. The reaction in the presence of Ag_2O in THF gave desired the 13-alkyloxy derivative (**11**) in 36.8% yield along with by-products such as the 15-alkoxymilbemycin (**12**, 19.5%), the 13-hydroxy derivative (**13**, 28.2%) and the 15-hydroxy derivative (**14**, 9.0%) as shown in Scheme 4. Replacement of Ag_2O with AgOTrf successfully resulted in decreasing the hydroxylated products (**13** and **14**), although no progress in the ratio of the 13-ether *vs.* the 15-ether was observed. A moderate improvement in the ratio was observed when mercury(II) iodide in THF was used as an catalyst. Better

Scheme 4.

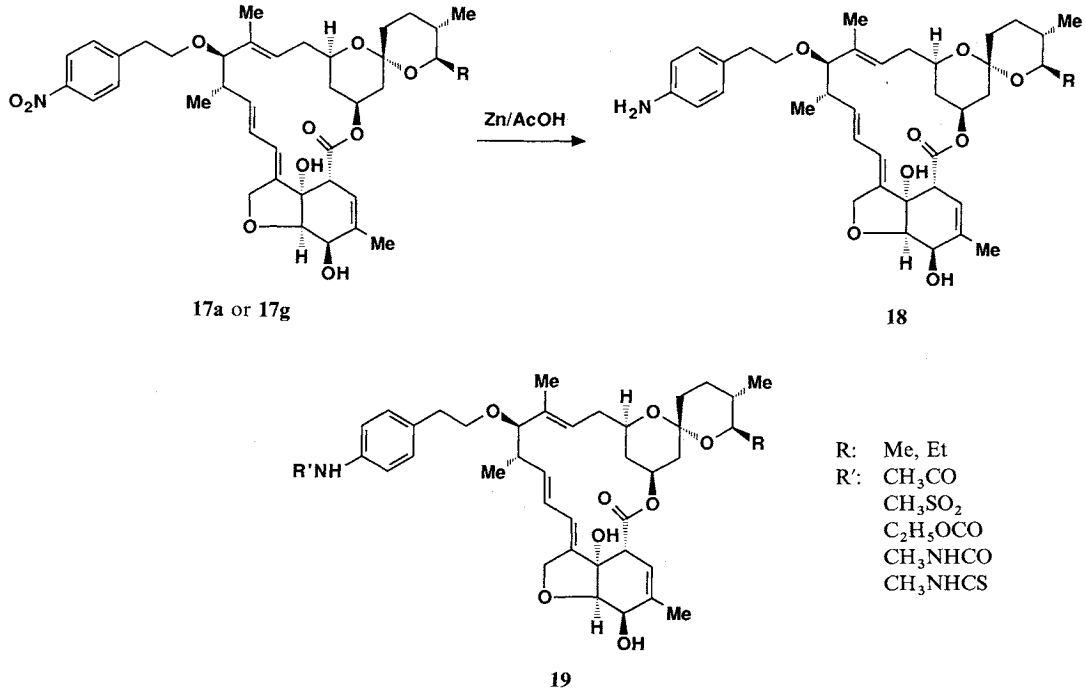


Scheme 5.



	R′	R
a	NO_2-C$_6$H$_4$-CH_2CH_2-	Et
b	Me	Et
c	*i*-Bu	Et
d	*t*-Bu	Et
e	C$_6$H$_{11}$-	Et
f	NO_2-C$_6$H$_4$-CH_2-	Et
g	NO_2-C$_6$H$_4$-CH_2CH_2-	Me

Scheme 6.



17a or **17g**

18

19

results in terms of both the yield and the regioselectivity were obtained when the reaction was carried out in the presence of HgI_2 in 1,2-dichloroethane. Most of the alcohols employed for the etherifications reacted smoothly with the 13-iodide (**15**) in the presence of mercury(II) iodide to give the corresponding 13-alkoxy milbemycins (**16**) in good yields. Addition of anhydrous $CaCO_3$ was more useful in reducing the moderate acidity caused by HI formed during the reaction thereby minimizing the decomposition of the product when prolonged reaction times were required. Alternatively, addition of 2,6-lutidine was found to accelerate this reaction, but lowered regioselectivity was also observed. The 13-alkyloxy-5-oxomilbemycins (**16**) thus obtained were converted to the 5β-hydroxy derivatives (**17**) by $NaBH_4$ reduction in methanol, and their anthelmintic activities were evaluated.

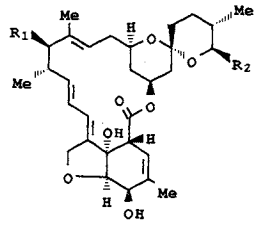
Some *N*-substituted 13-aminophenethyloxy derivatives (**19**) were prepared from the nitrophenethyloxy derivatives (**17a** or **17g**). Reduction with zinc dust in 90% acetic acid followed by treatment of the resulting aminophenethyloxy derivative (**18**) with a variety of electrophiles such as acyl halides gave *N*-substituted aminophenethyloxy derivatives (**19**).

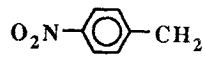
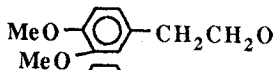
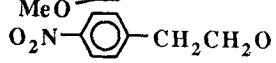
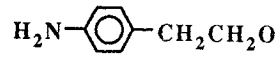
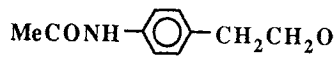
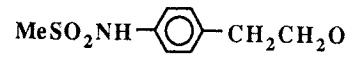
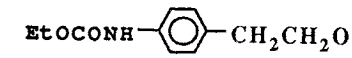
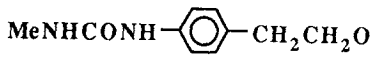
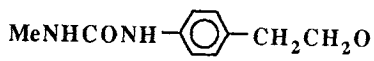
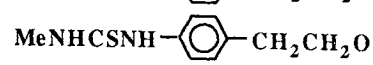
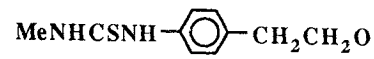
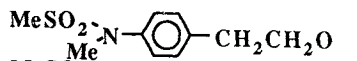
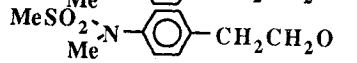
Biological Activity

The anthelmintic activities of a series of 13-alkyloxy derivatives of milbemycin A_4 and A_3 were assessed by oral administration to rat infected with *Nippostrongylus brasiliensis*.

The anthelmintic efficacy of the derivatives varied significantly with substituents at the 13-position, as shown in Table 2. The compounds with simple alkoxy residue exhibited only moderate activities. Yet, *N*-substituted aminophenethyloxy derivatives (**19**) were highly effective, some even surpassing ivermectin in the *in vivo* model system used. The milbemycin A_3 derivatives were significantly less active than the homologous A_4 compounds. Details of structure-activity relationship of these 13-alkyloxymilbemycins will

Table 2. Anthelmintic activity of selected 13-alkyloxymilbemycins.



Compound R_1	R_2	Efficacy (%) at dose rates	
		0.250 mg/kg	0.125 mg/kg
MeO	Et	44.0	49.5
i-BuO	Et	46.2	41.2
t-BuO	Et	84.8	62.8
	Et	40.8	28.5
	Et	76.0	12.8
	Et	99.8	98.1
	Et	87.0	82.4
	Et	98.5	92.3
	Et	96.7	68.1
	Et	100.0	100.0
	Et	99.6	99.6
	Et	100.0	93.3
	Me	—[a]	71.2
	Et	100.0	100.0
	Me	—[a]	63.6
	Et	100.0	99.7
	Me	—[a]	96.9
Milbemycin A_4		24.8	—[a]
Ivermectin		100.0	97.7

[a] Not tested.

be described in a separate publication.

Experimental

Products were purified by silica gel column (Wako CQ-3, 30~50 μm) and/or by reverse-phase high-performance liquid chromatography (HPLC) using YMC D-ODS-5 column. Purities of the products

were determined by analytical TLC on silica gel plates visualized by UV fluorescence and staining with 48% HBr, and by analytical HPLC on a YMC R-ODS-5 reverse phase column using UV absorption at 245~250 nm for detection. ^{1}H NMR spectra were recorded on JEOL JNM-GX270 instrument in $CDCl_3$ solution with Me_4Si as internal reference. Mass spectra were obtained on JEOL JMS-D300 mass spectrometer.

13β-Iodo-5-oxomilbemycin A_4 (**7**)

Method A

To a solution of 13β-hydroxy-5-oxomilbemycin A_4 (**6**, 557 mg, 1.0 mmol) in 1,2-dichloroethane (5 ml) were added pyridine (0.081 ml, 1.0 mmol) and trifluoromethanesulfonic anhydride (282 mg, 1.0 mmol) at 4°C and the mixture was stirred for 15 minutes at the same temperature. Powdered NaI (300 mg, 2.0 mmol) and dibenzo-18-crown-6 (20 mg) were added, and the resulting mixture was stirred at room temperature for 1.5 hours. The reaction mixture was diluted with EtOAc (50 ml), washed with water, 0.5 M citric acid, 5% $K_2S_2O_3$, 4% $NaHCO_3$, and with water successively, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc - hexane = 7 : 93). Recrystallization from EtOAc - hexane gave **7** (120 mg) as a pale yellow needles: MP 142°C (decomposition); ^{1}H NMR δ 0.84 (3H, d, J=6.6 Hz, C-24 CH_3), 0.99 (3H, t, J=6.3 Hz, C-25 CH_2CH_3), 1.23 (3H, d, J=6.6 Hz, C-12 CH_3), 1.54 (3H, s, C-14 CH_3), 1.89 (3H, s, C-4 CH_3), 2.65 (1H, m, C-12 H), 3.06 (1H, ddd, J=2.6, 9.2 and 9.2 Hz, C-25 H), 3.85 (1H, s, C-6 H), 3.99 (1H, s, C-7 OH), 4.58 (1H, d, J=11.0 Hz, C-13 H), 4.71 and 4.75 (2H, ABq, J=14.7 Hz, C-27 H), 6.53 (1H, dd, J=1.5 and 2.5 Hz, C-3 H); MS *m/z* 539 (M−I).

Anal Calcd for $C_{32}H_{43}O_7I$: C 57.66, H 6.50, I 19.04.
Found: C 57.42, H 6.48, I 19.49.

Method B

To a solution of 13-hydroxy-5-oxomilbemycin A_4 (**6**, 16.70 g, 30.0 mmol) in dichloromethane (75 ml) were added 2-chloroformyl-1,2,4-triazolo[4,3-*a*]pyridin-3-one (**9**, 5.93 g, 30.0 mmol) and pyridine (2.43 ml, 30.0 mmol), and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was filtered, and the filtrate was washed with 0.5 M citric acid, water, 4% $NaHCO_3$, and with water successively. The solution was dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give **10** as an amorphous solid; ^{1}H NMR δ 0.83 (3H, d, J=6.3 Hz, C-24 CH_3), 1.15 (3H, d, J=6.6 Hz, C-12 CH_3), 1.88 (3H, s, C-4 CH_3), 3.97 (1H, s, C-6 H), 4.57 (1H, d, J=11.0 Hz, C-13 H), 6.48 (H, dd, J=6.2 and 7.0 Hz, C-6′ H), 6.53 (1H, br s, C-3 H), 7.10 (1H, d, J=9.5 Hz, C-8′ H), 7.16 (1H, dd, J=6.2 and 9.5 Hz, C-7′ H), 7.71 (1H, d, J=7.0 Hz, C-5′ H). The urethane intermediate (**10**) was dissolved in 1,2-dichloroethane (300 ml), zinc iodide (51.4 g) was added, and the mixture was stirred at room temperature for 25 minutes. The reaction mixture was filtered, and the filtrate was washed with 10% $Na_2S_2O_3$, and then with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2 - hexane = 9 : 1). Recrystallization from benzene gave **7** (15.3 g) as pale yellow needles.

According to Method B, 13β-iodo-5-oxomilbemycin A_3 (**15**, R=CH_3) was prepared from 13β-hydroxy-5-oxomilbemycin A_3, as an amorphous solid; ^{1}H NMR δ 0.84 (3H, d, J=6.6 Hz, C-24 CH_3), 1.02 (3H, d, J=6.4 Hz, C-12 CH_3), 1.23 (3H, d, J=6.6 Hz, C-25 CH_3), 1.54 (3H, s, C-14 CH_3), 1.89 (3H, s, C-4 CH_3), 2.65 (1H, m, C-12 H), 3.20~3.30 (2H, m, C-25 and C-2 H), 3.95 (1H, s, C-6 H), 3.99 (1H, s, C-7 OH), 4.58 (1H, d, J=11.0 Hz, C-13 H), 6.53 (1H, m, C-3 H); MS *m/z* 525 (M−I).

Reaction of the 13-Iodomilbemycin (**7**) with Benzyl Alcohol in the Presence of Ag_2O

13β-Iodo-5-oxomilbemycin A_4 (**7**, 100 mg, 0.15 mmol) was dissolved in THF (2 ml), and benzyl alcohol (0.2 ml) and silver oxide (300 mg) were added. After being stirred at room temperature for 2 hours, the mixture was diluted with EtOAc (10 ml) and then filtered to remove insoluble materials. The filtrate was washed with 10% $Na_2S_2O_3$, and then with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc - hexane = 3 : 7) to give 13-benzyloxy-5-oxo-milbemycin (54.7 mg, 55.0%) as an amorphous solid; ^{1}H NMR δ 0.84 (3H, d, J=6.6 Hz, C-24 CH_3), 1.00 (3H, t, J=7.4 Hz, C-25 CH_2CH_3), 1.12 (3H, d, J=6.6 Hz, C-12 CH_3), 1.55 (3H, s, C-14

CH_3), 1.89 (3H, br s, C-4 CH_3), 2.57 (1H, m, C-12 H), 3.09 (1H, m, C-25 H), 3.34 (1H, d, J=9.9 Hz, C-13 H), 3.85 (1H, s, C-6 H), 3.92 (1H, s, C-7 OH), 4.20 and 4.45 (2H, ABq, J=12.0 Hz, $PhCH_2$), 4.74 (2H, br s, C-27 H), 6.54 (1H, dd, J=1.5 and 2.5 Hz, C-3 H), 7.25~7.40 (5H, m, Ph H); MS m/z 648 (M, $C_{39}H_{52}O_8$).

13-[2-(3,4-Dimethoxyphenyl)ethyloxy]milbemycin A_4 (**11**, R=3,4-di-methoxyphenyl ethyl)

Method A

3β-Iodo-5-oxomilbemycin A_4 (**7**, 333 mg, 0.50 mmol) was dissolved in THF (5 ml), and 3,4-dimethoxyphenethyl alcohol (991 mg, 5.0 mmol) and silver oxide (1.0 g) were added. After stirred at room temperature for 1 hour, the mixture was diluted with EtOAc (30 ml) and then filtered to remove insoluble materials. The filtrate was washed with 10% $Na_2S_2O_3$, and then with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in methanol (12 ml), and the solution was cooled to 4°C. After sodium borohydride (18 mg) had been added, the solution was stirred at 4°C for 30 minutes. The reaction mixture was diluted with EtOAc (50 ml), washed twice with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on an ODS column. Eluents with 70% CH_3CN were collected separately into four fractions. The first fraction was 15-hydroxy-$\Delta^{13,14}$-milbemycin A_4 (**14**, 25 mg); ^{1}H NMR δ 0.82 (3H, d, J=6.8 Hz, C-24 CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.08 (3H, d, J=6.9 Hz, C-12 CH_3), 1.59 (3H, s, C-14 CH_3), 1.88 (3H, s, C-4 CH_3), 2.99 (1H, m, C-25 H), 3.09 (1H, m, C-12 H), 3.26 (1H, m, C-2 H), 4.03 (1H, d, J=6.3 Hz, C-6 H), 4.08 (1H, dd, J=4.8 and 11.2 Hz, C-15 H), 4.30 (1H, m, C-5 H), 4.90 (1H, m, C-19 H), 5.15 (1H, d, J=9.3 Hz, C-13 H); MS m/z 558 (M, $C_{32}H_{46}O_8$). The second fraction, after removal of the solvent, gave 13-hydroxymilbemycin A_4 (**13**, 79 mg); ^{1}H NMR δ 0.83 (3H, d, J=6.5 Hz, C-24 CH_3), 0.99 (3H, t, J=7.4 Hz, C-25 CH_2CH_3), 1.13 (3H, d, J=6.5 Hz, C-12 CH_3), 1.59 (3H, s, C-14 CH_3), 1.88 (3H, s, C-4 CH_3), 2.37 (1H, m, C-12 H), 3.07 (1H, m, C-25 H), 3.27 (1H, q, J=2.2 Hz, C-2 H), 3.72 (1H, d, J=9.3 Hz, C-13 H), 3.97 (1H, d, J=6.2 Hz, C-6 H), 4.30 (1H, d, J=6.2 Hz, C-5 H), 4.68 and 4.71 (2H, ABq, J=14.5 Hz, C-27 H), 5.24 (1H, dd, J=4.0 and 8.5 Hz, C-15 H); MS m/z 558 (M, $C_{32}H_{46}O_8$). The next fraction contained 15-[2-(3,4-dimethoxyphenyl)ethyloxy]-$\Delta^{13,14}$-milbemycin A_4 (**12**, 70 mg); ^{1}H NMR δ 0.83 (3H, d, J=6.2 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.07 (3H, d, J=6.6 Hz, C-12 CH_3), 1.44 (3H, s, C-14 CH_3), 1.88 (3H, s, C-4 CH_3), 2.79 (2H, m, $PhCH_2$) 2.95 (1H, m, C-25 H), 3.09 (1H, m, C-12 H), 3.26 (1H, m, C-2 H), 3.57 (1H, dd, J=4.0 and 11.0 Hz, C-15 H), 3.80 and 3.87 (2×3H, two s, OCH_3), 4.03 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br s, C-5 H), 4.86 (1H, m, C-19 H), 5.10 (1H, d, J=9.2 Hz, C-13 H). The desired product (**11**, 133 mg) was obtained, after removal of the solvent from the last fraction, as an amorphous solid; ^{1}H NMR δ 0.82 (3H, d, J=6.2 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.07 (3H, d, J=6.6 Hz, C-12 CH_3), 1.51 (3H, s, C-14 CH_3), 1.87 (3H, s, C-4 CH_3), 2.79 (2H, m, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.22 (1H, d, J=9.5 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.86 and 3.87 (2×3H, two s, OCH_3), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br d, C-5 H), 6.73 (1H, d, J=8.8 Hz, Ph H), 6.75 (1H, s, Ph H), 6.79 (1H, d, J=8.8 Hz, Ph H).

13β-[2-(4-Nitrophenyl)ethyloxy]milbemycin A_4 (**17a**, R'=4-nitrophenethyl, R=Et)

Step A

To a solution of 3β-iodo-5-oxomilbemycin A_4 (**15a**, R=Et) (1.00 g, 1.50 mmol) in 1,2-dichloroethane (6.0 ml) were added 4-nitrophenethyl alcohol (1.250 g, 7.5 mmol), anhydrous $CaCO_3$ (300 mg) and mercury(II) iodide (1.25 g, 7.5 mmol), and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc (50 ml) and then filtered to remove insoluble materials. The filtrate was washed with 20% KI, 10% $Na_2S_2O_3$, and with water successively, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc-hexane=15:85) to give 0.774 g of 5-oxo-13β-[2-(4-nitrophenyl)ethyloxy]milbemycin A_4 (**16a**, R'=4-nitrophenethyl, R=Et) as an amorphous solid; ^{1}H NMR δ 0.83 (3H, d, J=6.8 Hz, C-24 CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.01 (3H, d, J=6.8 Hz, C-12 CH_3), 1.43 (3H, s, C-14 CH_3), 1.89 (3H, br s, C-4 CH_3), 2.94 (2H, t, J=6.3 Hz, $PhCH_2$), 3.06 (1H, m, C-25 H), 3.20 (1H, d, J=9.8 Hz, C-13 H), 3.84 (1H, s, C-7, OH), 3.93 (1H, s, C-6 H), 6.54 (1H, m, C-3 H), 7.37 (2H, d, J=8.8 Hz, Ph H), 8.14 (2H, d, J=8.8 Hz, Ph H).

Step B

The 5-oxo derivative (**16a**, 0.710 g), prepared in step A, was dissolved in methanol (27 ml), and the solution was cooled to 4°C. After sodium borohydride (18 mg) had been added, the solution was stirred at 4°C for 20 minutes. The reaction mixture was diluted with EtOAc (100 ml), washed twice with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc-hexane=2:8) to give 0.693 g of 13β-[2-(4-nitrophenyl)ethyloxy]-milbemycin A_4 (**17a**, R'=4-nitrophenethyl, R=Et) as an amorphous solid; ^{1}H NMR δ 0.82 (3H, d, J=6.6 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.05 (3H, d, J=6.6 Hz, C-12 CH_3), 1.44 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.75 (2H, m, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.95 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br d, C-5 H), 5.40 (1H, m, C-3 H), 6.74 (2H, d, J=8.2 Hz, Ph H), 7.02 (2H, d, J=8.2 Hz, Ph H); MS m/z 707 (M, $C_{40}H_{53}NO_{10}$).

Similarly, Compounds **17b**~**17i** were prepared from **15a** or **15b** (R=Me) with corresponding alcohols according to the procedure described for **17a**.

13β-Methoxymilbemycin A_4 (**17b**, R'=Me, R=Et)

Yield 76.2%; ^{1}H NMR δ 0.83 (3H, d, J=6.2 Hz, C-24 CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.09 (3H, d, J=6.2 Hz, C-12 CH_3), 1.48 (3H, s, C-14 CH_3), 1.88 (3H, s, C-4 CH_3), 3.09 (1H, m, C-25 H), 3.10 (1H, d, J=9.9 Hz, C-13 H), 3.16 (3H, s, OCH_3), 3.27 (1H, m, C-2 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 3.99 (1H, s, C-7 OH), 4.29 (1H, br d, C-5 H), 5.40 (1H, br s, C-3 H); MS m/z 572 (M, $C_{33}H_{48}O_8$).

13β-(2-Methylpropyloxy)milbemycin A_4 (**17c**, R'=*i*-Bu, R=Et)

Yield 81.3%; ^{1}H NMR δ 0.82 (3H, d, J=6.4 Hz, C-24 CH_3), 0.88 (3H, d, J=5.4 Hz, *i*-Bu CH_3), 0.89 (3H, d, J=5.4 Hz, *i*-Bu CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.10 (3H, d, J=6.3 Hz, C-12 CH_3), 1.48 (3H, s, C-14 CH_3), 1.88 (3H, s, C-4 CH_3), 2.85 (1H, dd, J=6.6 and 9.0 Hz, C-13 OCH), 3.08 (1H, dd, J=6.4 and 9.0 Hz, C-13 OCH), 3.17 (1H, d, J=9.8 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.96 (1H, d, J=6.4 Hz, C-6 H), 4.00 (1H, br s, C-7 OH), 4.29 (1H, br d, C-5 H), 5.41 (1H, br s, C-3 H); MS m/z 614 (M, $C_{36}H_{54}O_8$).

13β-(Tertiary Butyloxy)milbemycin A_4 (**17d**, R'=*t*-Bu, R=Et)

Yield 55.7%; ^{1}H NMR δ 0.82 (3H, d, J=6.4 Hz, C-24 CH_3), 1.00 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.04 (3H, d, J=6.3 Hz, C-12 CH_3), 1.16 (9H, s, *t*-Bu), 1.55 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 3.07 (1H, ddd, J=2.9, 6.3 and 6.3, C-25), 3.27 (1H, m, C-2 H), 3.57 (1H, d, J=9.8 Hz, C-13 H), 3.95 (1H, d, J=5.9 Hz, C-6 H), 3.98 (1H, br s, C-7 OH), 4.29 (1H, br t, J=6.3 Hz, C-5 H), 5.41 (1H, br s, C-3 H); MS m/z 614 (M, $C_{36}H_{54}O_8$).

13β-Cyclohexyloxymilbemycin A_4 (**17e**, R'=cyclohexyl, R=Et)

Yield 83.8%; ^{1}H NMR δ 0.83 (3H, d, J=6.3 Hz, C-24 CH_3), 0.99 (3H, t, J=7.4 Hz, C-25 CH_2CH_3), 1.08 (3H, d, J=6.6 Hz, C-12 CH_3), 1.50 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 3.06 (1H, m, C-25), 3.15 (1H, m, C-13 OCH), 3.26 (1H, m, C-2 H), 3.30 (1H, d, J=8.7 Hz, C-13 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.28 (1H, br s, C-5 H), 5.40 (1H, br s, C-3 H); MS m/z 640 (M, $C_{38}H_{56}O_8$).

13-(4-Nitrobenzyloxy)milbemycin A_4 (**17f**, R'=4-nitrobenzyl, R=Et)

Yield 61.0%; ^{1}H NMR δ 0.83 (3H, d, J=6.6 Hz, C-24 CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.14 (3H, d, J=6.2 Hz, C-12 CH_3), 1.54 (3H, s, C-14 CH_3), 1.88 (3H, br s, C-4 CH_3), 2.48 (1H, m, C-12 H), 3.07 (1H, m, C-25), 3.27 (1H, m, C-2 H), 3.36 (1H, d, J=9.9 Hz, C-13 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 3.97 (1H, s, C-7 OH), 4.30 and 4.52 (2H, ABq, J=13.2 Hz, $PhCH_2$), 5.40 (1H, br s, C-3 H), 7.47 (2H, d, J=8.8 Hz, Ph H), 8.20 (2H, d, J=8.8 Hz, Ph H); MS m/z 693 (M, $C_{39}H_{51}NO_{10}$).

13-(4-Nitrophenethyloxy)milbemycin A_3 (**17g**, R'=4-nitrophenethyl, R=Me)

Yield 75.4%; ^{1}H NMR δ 0.83 (3H, d, J=6.3 Hz, C-24 CH_3), 1.02 (3H, d, J=6.8 Hz, C-12 CH_3), 1.14 (3H, d, J=6.3 Hz, C-25 CH_3), 1.43 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.75 (2H, m, $PhCH_2$), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.20~3.30 (2H, m, C-25 and C-2 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br s, C-5 H), 5.41 (1H, m, C-3 H), 6.74 (2H, d, J=8.2 Hz, Ph H), 7.02 (2H, d, J=8.2 Hz, Ph

H); MS *m/z* 693 (M, $C_{39}H_{51}NO_{10}$).

13-{2-[4-(*N*-Methanesulfonylmethylamino)phenyl]ethyloxy}milbemycin A_4 (**17h**, R′=4-(*N*-methanesulfonylmethylamino)phenethyl, R=Et)

Yield 62.1%; ^{1}H NMR δ 0.82 (3H, d, J=6.4 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.02 (3H, d, J=6.3 Hz, C-12 CH_3), 1.42 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.82 (3H, s, SO_2CH_3), 3.08 (1H, m, C-25), 3.21 (1H, d, J=9.8 Hz, C-13 H), 3.26 (1H, m, C-2 H), 3.30 (3H, s, NCH_3), 3.95 (1H, d, J=6.4 Hz, C-6 H), 4.29 (1H, d, J=6.4 Hz, C-5 H), 5.40 (1H, br s, C-3 H), 7.22 (2H, dd, J=2.4 and 6.4 Hz, Ph H), 7.28 (2H, dd, J=2.4 and 6.4 Hz, Ph H); MS *m/z* 769 (M, $C_{42}H_{59}NO_{10}S$).

13-{2-[4-(*N*-Methanesulfonylmethylamino)phenyl]ethyloxy}milbemycin A_3 (**17i**, R′=4-(*N*-methanesulfonylmethylamino)phenethyl, R=Me)

Yield 70.8%; ^{1}H NMR δ 0.83 (3H, d, J=6.3 Hz, C-24 CH_3), 1.02 (3H, d, J=6.8 Hz, C-12 CH_3), 1.14 (3H, d, J=6.3 Hz, C-25 CH_3), 1.42 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.81 (3H, s, SO_2CH_3), 3.20 (1H, d, J=9.8 Hz, C-13 H), 3.20~3.30 (2H, m, C-25 and C-2 H), 3.30 (3H, s, NCH_3), 3.95 (1H, d, J=5.9 Hz, C-6 H), 4.00 (1H, s, C-7 OH), 4.28 (1H, br d, C-5 H), 5.39 (1H, br s, C-3 H), 7.22 (2H, dd, J=2.4 and 6.5 Hz, Ph H), 7.28 (2H, d, J=2.4 and 6.5 Hz, Ph H); MS *m/z* 755 (M, $C_{41}H_{57}NO_{10}S$).

13-[2-(4-Aminophenyl)ethyloxy]milbemycin A_4 (**18a**, R=Et)

To a solution of the nitrophenethyloxy derivative (**17a**, 708 mg, 1.00 mmol) in 90% acetic acid was added zinc powder (700 mg), while cooling with water, and the mixture was stirred for 20 minutes. The reaction mixture was diluted with EtOAc (50 ml) and filtered to remove insoluble materials. The filtrate was washed three times with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (ODS, 75% CH_3CN) to give **17a** (622 mg, 91.7%) as amorphous solid; ^{1}H NMR δ 0.82 (3H, d, J=6.6 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.05 (3H, d, J=6.6 Hz, C-12 CH_3), 1.44 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.75 (2H, m, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.95 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br d, J=6.8 Hz, C-5 H), 5.40 (1H, m, C-3 H), 6.74 (2H, d, J=8.2 Hz, Ph H), 7.02 (2H, d, J=8.2 Hz, Ph H).

13-[2-(4-Aminophenyl)ethyloxy]milbemycin A_3 (**18b**, R=Me)

Similar treatment of 13-(4-nitrophenethyloxy)milbemycin A_3 (**17g**) by the procedure described for **18a** gave **18b** (87%) as an amorphous solid; ^{1}H NMR δ 0.83 (3H, d, J=6.3 Hz, C-24 CH_3), 1.02 (3H, d, J=6.8 Hz, C-12 CH_3), 1.14 (3H, d, J=6.3 Hz, C-25 CH_3), 1.44 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.75 (2H, m, $PhCH_2$), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.20~3.30 (2H, m, C-25 and C-2 H), 3.95 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, br d, J=6.8 Hz, C-5 H), 5.41 (1H, m, C-3 H), 6.74 (2H, d, J=8.2 Hz, Ph H), 7.02 (2H, d, J=8.2 Hz, Ph H).

13-[2-(4-Acetamidophenyl)ethyloxy]milbemycin A_4 (**19a**, R′=acetyl, R=Et)

To a solution of **18a** (200 mg, 0.295 mmol) in dichloromethane (3.0 ml) were added pyridine (0.0253 ml, 0.33 mmol) and acetyl chloride (28.3 mg, 0.33 mmol), and the mixture was stirred at room temperature for 15 minutes. The reaction mixture was poured into ice-water and extracted with dichloromethane. The extract was washed with 1 N HCl, water, 4% Na_2CO_3, and water successively, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (ODS, 90% CH_3CN) to give **19a** (188 mg, 89%) as an amorphous solid; ^{1}H NMR δ 0.82 (3H, d, J=6.2 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.04 (3H, d, J=6.2 Hz, C-12 CH_3), 1.43 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.17 (3H, s, CH_3CO), 2.80 (2H, m, $PhCH_2$), 3.06 (1H, m, C-25 H), 3.20 (1H, d, J=9.9 Hz, C-13 H), 3.26 (1H, m, C-2 H), 3.95 (1H, s, C-6 H), 3.99 (1H, s, C-7 OH), 4.29 (1H, br s, C-5 H), 5.41 (1H, m, C-3 H), 7.11 (1H, br s, NH), 7.15 (2H, d, J=8.4 Hz, Ph H), 7.39 (2H, d, J=8.4 Hz, Ph H).

Similarly, following compounds, **19b** and **19c**, were prepared from **18a** by using chloroethyl formate or methanesulfonyl chloride instead of acetyl chloride.

13-[2-(4-Ethoxycarbonylaminophenyl)ethyloxy]milbemycin A_4 (**19b**, R′=ethoxycarbonyl, R=Et)

Yield 83.6%; ^{1}H NMR δ 0.82 (3H, d, J=6.6 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3),

1.04 (3H, d, J=6.6 Hz, C-12 CH_3), 1.31 (3H, t, J=7.0 Hz, $COOCH_2CH_3$), 1.43 (3H, s, C-14 CH_3), 1.83 (3H, s, C-4 CH_3), 2.79 (2H, m, $PhCH_2$), 3.06 (1H, m, C-25), 3.20, (1H, d, J=9.9 Hz, C-13 H), 3.26 (1H, m, C-2 H), 3.96 (1H, d, J=6.3 Hz, C-6 H), 3.99 (1H, s, C-6 H), 4.21 (2H, q, J=7.0 Hz, $COOCH_2$), 4.25 (1H, br t, C-5 H), 5.40 (1H, br s, C-3 H), 7.13 (2H, d, J=8.4 Hz, Ph H), 7.28 (2H, d, J=8.4 Hz, Ph H); MS m/z 749 (M, $C_{43}H_{59}NO_{10}$).

13-[2-(4-Methanesulfonylaminophenyl)ethyloxy]milbemycin A_4 (**19c**, R'=methanesulfonyl, R=Et)
Yield 89.4%; ^{1}H NMR δ 0.82 (3H, d, J=6.5 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.02 (3H, d, J=6.5 Hz, C-12 CH_3), 1.42 (3H, s, C-14 CH_3), 1.87 (3H, s, C-4 CH_3), 2.82 (2H, m, $PhCH_2$), 2.97 (3H, s, CH_3SO_2), 3.05 (1H, m, C-25 H), 3.20 (1H, d, J=9.8 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.96 (1H, d, J=6.4 Hz, C-6 H), 4.29 (1H, br d, J=5.8 Hz, C-5 H), 5.40 (1H, br s, C-3 H), 6.24 (1H, s, NH), 7.13 (2H, d, J=8.5 Hz, Ph H), 7.21 (2H, d, J=8.5 Hz, Ph H); MS m/z 755 (M, $C_{41}H_{57}NO_{10}S$).

13-{2-[4-(3-Methylureido)phenyl]ethyloxy}milbemycin A_4 (**19d**, R'=methylureido, R=Et)
To a solution of **18a** (200 mg, 0.295 mmol) in THF (2.0 ml) was added methylisocyanate (20.5 mg, 0.36 mmol) and the mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated *in vacuo*. The residue was purified by column chromatography (ODS, 70% CH_3CN) to give **19d** (196 mg, 90.5%) as an amorphous solid; ^{1}H NMR δ 0.82 (3H, d, J=6.6 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.03 (3H, d, J=6.8 Hz, C-12 CH_3), 1.43 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.83 (3H, d, J=4.8 Hz, NCH_3), 3.05 (1H, m, C-25 H), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.00 (1H, s, C-7 OH), 4.29 (1H, br t, J=6.6 Hz, C-5 H), 5.40 (1H, m, C-3 H), 6.21 (1H, s, NH), 7.17 (4H, m, Ph H); MS m/z 703 ($M-CH_3NH_2$).

Similarly, compounds **19e**~**19g** were prepared from **18a** or **18b** by using methyl isocyanate or methyl isothiocyanate according to the procedure described for **19d**.

13-{2-[4-(3-Methylureido)phenyl]ethyloxy}milbemycin A_3 (**19e**, R'=methylureido, R=Me)
Yield 82.6%; ^{1}H NMR δ 0.83 (3H, d, J=6.8 Hz, C-24 CH_3), 1.02 (3H, d, J=6.3 Hz, C-12 CH_3), 1.14 (3H, d, J=6.9 Hz, C-25 CH_3), 1.43 (3H, s, C-14 CH_3), 1.83 (3H, s, C-4 CH_3), 3.20 (1H, d, J=9.7 Hz, C-13 H), 3.20~3.30 (2H, m, C-25 H and C-2 H), 3.95 (1H, d, J=6.4 Hz, C-6 H), 4.01 (1H, s, C-7 OH), 4.29 (1H, br t, C-5 H), 4.72 (1H, d, J=4.9 Hz, NH), 5.39 (1H, br s, C-3 H), 7.13~7.21 (4H, m, Ph H); MS m/z 689 ($M-CH_3NH_2$).

13-{2-[4-(3-Methylthioureido)phenyl]ethyloxy}milbemycin A_4 (**19f**, R'=methylthioureido, R=Et)
Yield 80.2%; ^{1}H NMR δ 0.82 (3H, d, J=6.2 Hz, C-24 CH_3), 0.98 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.06 (3H, d, J=6.6 Hz, C-12 CH_3), 1.42 (3H, s, C-14 CH_3), 1.87 (3H, br s, C-4 CH_3), 2.85 (2H, t, $PhCH_2$), 3.04 (1H, m, C-25 H), 3.13 (3H, d, J=4.8 Hz, NCH_3), 3.21 (1H, d, J=9.9 Hz, C-13 H), 3.27 (1H, m, C-2 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 3.99 (1H, s, C-7 OH), 4.29 (1H, br t, C-5 H), 5.40 (1H, m, C-3 H), 5.94 (1H, br s, NH), 7.11 (2H, d, J=8.4 Hz, Ph H), 7.28 (2H, d, J=8.4 Hz, Ph H); MS m/z 719 ($M-CH_3NH_2$).

13-{2-[4-(3-Methylthioureido)phenyl]ethyloxy}milbemycin A_3 (**19g**, R'=methylthioureido, R=Me)
Yield 56.4%; ^{1}H NMR δ 0.83 (3H, d, J=6.4 Hz, C-24 CH_3), 1.01 (3H, d, J=6.4 Hz, C-12 CH_3), 1.14 (3H, d, J=6.4 Hz, C-25 CH_3), 1.61 (3H, s, C-14 CH_3), 1.87 (3H, s, C-4 CH_3), 2.85 (2H, m, $PhCH_2$), 3.12 (3H, d, J=4.4 Hz, NCH_3), 3.20 (1H, d, J=9.8 Hz, C-13 H), 3.20~3.30 (2H, m, C-25 H and C-2 H), 3.95 (1H, d, J=6.4 Hz, C-6 H), 3.99 (1H, s, C-7 OH), 4.29 (1H, br t, C-5 H), 5.39 (1H, br s, C-3 H), 5.94 (1H, br s, NH), 7.11 (2H, d, J=7.8 Hz, Ph H), 7.27 (2H, d, J=7.8 Hz, Ph H), 7.61 (1H, s, NH); MS m/z 705 ($M-CH_3NH_2$).

Anthelmintic Assays

The anthelmintic activity against *Nippostrongylus brasiliensis*, a nematode parasitic to rats, was examined with groups each containing 3 Wistar strain rats of body weight in the range from 40 to 60 g. The rats were infested percutaneously with about 100 larvae of the nematode for each rat. Solutions containing the test compounds at various concentrations were administered orally 3 days after the infection.

Each solution was prepared by dissolving 1.0 mg of the test compound in 0.1 ml of dimethylformamide, and then adding 10 ml of polyethylene glycol (PEG 400) to the solution. The solution was then adjusted by the addition of PEG 400 to achieve a concentration of 0.250 or 0.125 mg/kg. The rats were killed 4 days after the infection, and the number of parasites in the small intestine were counted.

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