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Anthelmintic Activity of 13-Alkoxy Milbemycin Derivatives

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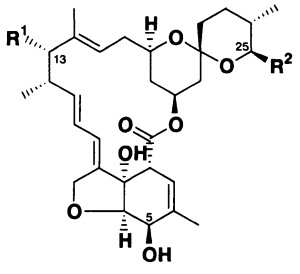
A number of 13-alkoxy milbemycin derivatives were synthesized to evaluate their anthelmintic activity. We report the strategy for developing a potent anthelmintic product, 13-[4-(*N*-methanesulfonyl-*N*-methylamino)-phenylethyloxy]milbemycin. The details of the structure-activity relationships of those derivatives are also discussed.

Milbemycin derivatives are known as potent antiparasitic agents and have attracted a great deal of interest recently[1]. On the other hand, ivermectin, which is widely used as a potent parasiticide for livestock[2], is known to possess similar activity to milbemycins against endoparasites. The structures of the milbemycin derivatives and ivermectin closely resemble each other, having a 16-membered ring (Fig. 1), although ivermectin has higher activity than milbemycins.

This difference in the activity is due to the substituent at the 25-position. It is known that the lipophilicity on the substituent at the 25-position contributes to their anthelmintic activity (Table 1). Thus it seemed easier to synthesize the ivermectin derivatives than milbemycin derivatives to find more active products. However, owing to availability of substrates, we decided to develop new milbemycin derivatives, which have as strong activity as ivermectin. As it is also known that the substituent at the 13-position strongly influences the activity[3], a great number of substituents at the 13-position, which overcome the disadvantage of the substituent at the 25-position, have been examined. Evaluations of 13-halomilbemycin[4,5], 13-alkylmilbemycin[6,7], and 13-acyloxymilbemycin[8,9] have already been reported. According to those studies, the 13-acyloxymilbemycins have quite strong activity and are thus very attractive, but the ester bond seems to be susceptible to esterase *in vivo*, producing the inactive 13-hydroxymilbemycin. That is why we aimed our research at 13-alkoxymilbemycins, which have an ether bond in place of the susceptible ester bond at the 13-position. The 13-alkoxymilbemycins also turned out to have strong anthelmintic activity, as reported in our previous

Fig. 1. The structures of milbemycins and ivermectin.



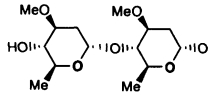
	R[1]	R[2]
Milbemycin A3	**H**	**Me**
Milbemycin A4	**H**	**Et**
Ivermectin		**mixture of s-Bu and i-Pr**

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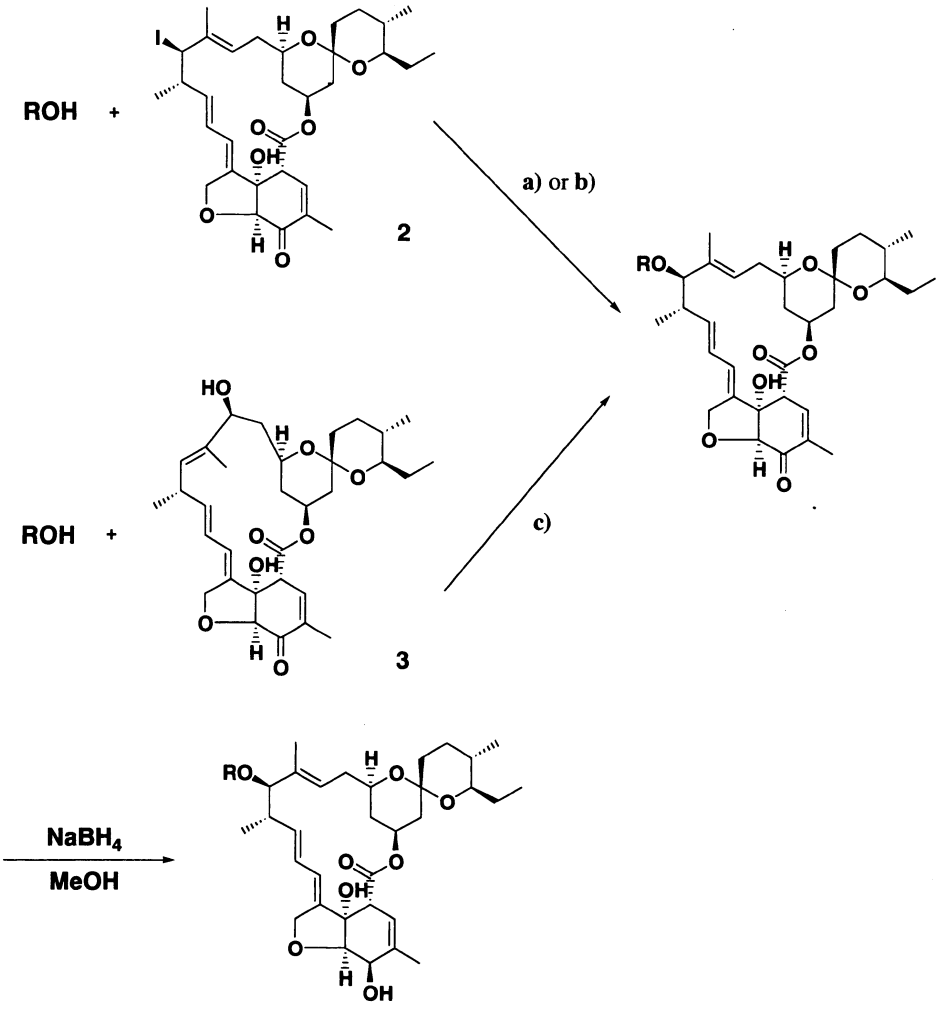
Table 1. The relationships between the lipophilicity on the substituent at the 25-position (R^2) and activity.

R^1	R^2	Dose (mg/kg)	Percent inhibition of growth of *N. brasiliensis* in the rats.
H	Me	1	47.3%
H	Et	1	81.8%
H	i-Pr	1	97.6%
H	s-Bu	0.25	86.7%

Scheme 1.



a) HgI_2, 2,6-Lutidine, Dichloroethane, b) Ag_2O, Dichloroethane, c) CuI, CF_3SO_3H, Dichloromethane

paper[10]. Following this research, further detail of the structure-activity relationships was examined and 13-(4-substituted phenyletyloxy)milbemycins, especially 13-[4-(*N*-methanesulfonyl-*N*-methylamino)-phenylethyloxy]-milbemycin (**1**), proved to possess considerably high and efficient activity. That is, we finally found an ideal derivative.

We report the strategy for developing the series of

derivatives to find an efficient and effective product and also describe the details of their structure-activity relationships.

Chemistry

Using our method already reported in previous papers[10,11], a number of derivatives was synthesized from milbemycin A_4 *via* 13-iodomilbemycin (**2**)[10] or 15-hydroxy-5-oxomilbemycin (**3**)[11] (Scheme 1).

Biological Results and Discussion

The activity of this series of 13-alkoxymilbemycin derivatives was evaluated by oral administration to rats infected with *Nippostrongylus brasiliensis* by use of the method described in the previous paper.

Firstly, the length of the carbon chain between the benzene ring and the oxygen atom (Fig. 2, position A) was examined (Table 2). Those results showed that the efficacy was the strongest when the number of the carbon chain was two (**4**, **5**, **6**). The efficacy clearly decreased when the chain was shorter or longer. In addition, this tendency was not dependent on the substituent at the benzene ring. Thus, the length of the carbon chain was fixed as two.

Secondly, substitution on the carbon chain was examined (Table 3). When the polar substituent was on the carbon chain, the efficacy decreased (**7**, **8**). When the chain had a ring formation (**9**, **10**), the efficacy did not change much compared to the straight chain. Regarding its availability and its facility in synthesis, the straight chain was chosen as a candidate.

Thirdly, the position of the substituent on the benzene ring (Fig. 2, position B) was examined (Table 4). The activity of the derivatives was maximized when the substituent on the benzene ring was at the *p*-position (**4**, **11**). An amino (or substituted amino) group was chosen as a candidate to provide a greater number of potential derivatives for pursuing an ideal compound, although there did not seems to much difference in activity between the

Fig. 2. The examined position in the milbemycin derivative.

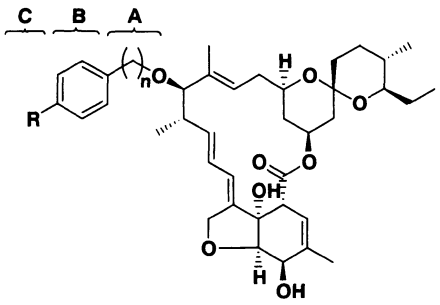


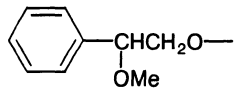
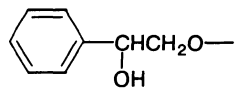
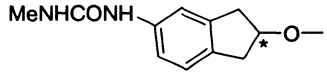
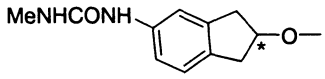
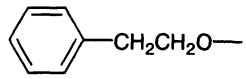
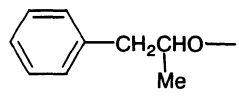
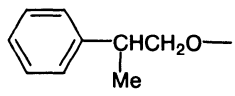
Table 2. Antiparasitic acitivity of derivatives which vary in the length of the carbon chain at position A in Fig. 2.

Compound	Structure	n	Efficacy (%)* at dose rates		
			0.25mg/kg	0.125mg/kg	0.063mg/kg
12	H_2N—Ph—$(CH_2)_nO$—	1	NT	0.4	12.3
4		2	98.5	92.3	NT
13		3	NT	32.8	38.7
14	MeO, MeO—Ph—$(CH_2)_nO$—	1	61.2	0	NT
5		2	99.8	98.1	NT
15		3	NT	8.5	0
16	AcNH—Ph—$(CH_2)_nO$—	1	15.1	7.8	4.3
6		2	96.7	68.1	NT
17		3	NT	59.5	27.9

* : Percent inhibition of growth of *N. brasiliensis* in rats.

NT: not tested.

Table 3. Antiparasitic activity of derivatives which vary in the substituent at position A in Fig. 2.

Compound	Structure	Efficacy (%)* at dose rates	
		0.25mg/kg	0.125mg/kg
7		82.3	29.9
8		27.2	16.8
9	 isomer A	NT	95.9
10	 isomer B	NT	98.6
18		73.5	44.3
19		86.7	27.8
20		98.4	81.6

* : Percent inhibition of growth of *N. brasiliensis* in rats.

NT: not tested.

Table 4. Antiparasitic activity of derivatives which vary in the position of the substituent on the benzene ring at position B in Fig. 2.

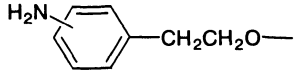
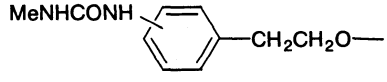
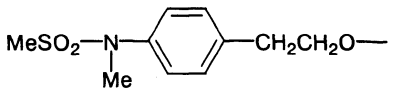
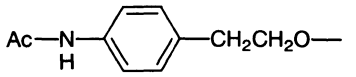
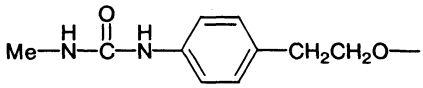
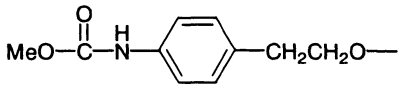
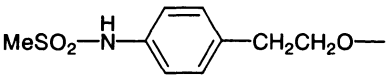
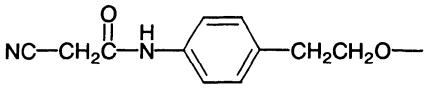
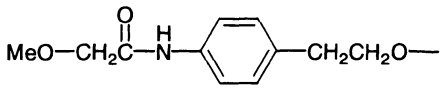
Percent inhibition of growth of *N.brasiliensis* in rats at a dose of 0.25 mg/kg			
Structure	Position		
	o-	*m-*	*p-*
	**21**	**22**	**4**
	33.8	0	98.5
	**23**	**24**	**11**
	49	85.6	98.6

Table 5. Antiparasitic activity of derivatives which vary in the substituent on the benzene ring at position C in Fig. 2.

	Structure	Efficacy (%)* at dose rates		
		0.125mg/kg	0.063mg/kg	0.032mg/kg
1		100	99	NT
6		68.1	NT	NT
11		93.3	89.0	66.4
25		99.7	94.5	80.3
26		100	90.7	87.8
27		100	98.9	94.6
28		92.8	66.9	NT

* : Percent inhibition of growth of *N. brasiliensis* in rats.

NT: not tested.

amino group and the methoxy group (Table 2, compound **4** *versus* **5**).

Lastly, the various *N*-substituted aminophenylethyloxy derivatives were examined (Fig. 2, position C) (Table 5). From the evaluation, *N*-methanesulfonyl-*N*-methylamino-phenylethyloxymilbemycin (**1**) turned out to possess considerably high and efficient activity.

Experimental

^{1}H NMR spectra were recorded on a JNM GSX-400 spectrometer using TMS as the internal standard. Mass spectra were obtained on a JOEL FABmate.

4-(*N*-Methanesulfonyl-*N*-methylamino)phenethyl Alcohol (**1**)

(1) 4-Aminophenylethyloxy-*t*-butyldimethylsilane

4-Nitrophenethyl alcohol (10.02 g, 60 mmol) was dissolved in DMF (70 ml), imidazole (5.44 g, 80 mmol) and *t*-butyldimethylsilyl chloride (12.08 g, 80 mmol) were added and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc (500 ml) and washed with H_2O twice, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in 90% AcOH (300 ml), then the solution was cooled to 4°C and zinc dust (30 g) was added. The mixture was stirred at room temperature for 20 minutes, and then the mixture was diluted with EtOAc (700 ml) and filtered. The filtrate was washed with H_2O twice, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue

was chromatographed on silica gel with the eluent (EtOAc : cyclohexane=1 : 3) to obtain 4-aminophenylethyloxy-*t*-butyl dimethyl silane (12.55 g, 83.2% yield).

(2) 4-(*N*-Methansulfonyl-*N*-methylamino)phenethyl Alcohol

4-Aminophenylethyloxy *t*-butyldimethyl silane was dissolved in 1,2-dichloroethane (20 ml), pyridine (2.0 ml) and methanesulfonyl chloride (1.63 ml, 21 mmol) were added. The mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc, then washed with 1 N-HCl, H_2O, 4% $NaHCO_3$, and H_2O again, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in *N*-methylpyrolidone (100 ml), iodomethane (1.56 ml, 25 mmol) and sodium hydride (55%, 873 mg, 20 mmol) were added, and the mixture was stirred at room temperature for 3 hours. The reaction mixture was poured into cold diluted HCl, extracted with EtOAc, and washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in MeOH (50 ml), *p*-toluenesulfonic acid monohydrate (50 mg) was added and the mixture was stirred at room temperature for 20 minutes. The reaction mixture was diluted with EtOAc (200 ml), washed with 4% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was crystallized from EtOAc and hexane to obtain 2-[4-(*N*-methansulfonyl-*N*-methylamino)phenyl]ethanol (1.61 g, 58.8% yield).

13-[4-(*N*-Methanesulfonyl-*N*-methylamino)phenylethyloxy]-5-hydroxymilbemycin (**1**)

(1) 13-[4-(*N*-Methansulfonyl-*N*-methylamino)phenylethyloxy]-5-oxomilbemycin

4-(*N*-Methansulfonyl-*N*-methylamino)phenethyl alcohol (2.75 g, 12 mmol) was dissolved in dichloromethane (25 ml), copper(I) iodide (480 mg, 2.52 mmol), trifluoromethanesulfonic acid (0.35 ml, 4.0 mmol) and 15-hydroxy-5-oxomilbemycin (1.36 g, 2.5 mmol)[11] were added, and the mixture was stirred at room temperature for 25 minutes. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with H_2O twice, 4% $NaHCO_3$, and H_2O again, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. To remove the remaining 4-(*N*-methansulfonyl-*N*-methylamino)phenethyl alcohol as a crystal, the residue was crystallized from EtOAc and cyclohexane and filtered. Then the filtrate was chromatographed on silica gel with the eluent (EtOAc : cyclohexane=35 : 65) to obtain 13-[4-(*N*-methansulfonyl-*N*-methylamino)phenylethyloxy]-5-oxomilbemycin (1.79 g, 95.2% yield).

(2) 13-[4-(*N*-Methanesulfonyl-*N*-methylamino)phenylethyloxy]-5-hydroxymilbemycin

Sodium borohydride (1.20 g) was dissolved in a mixture of THF (50 ml) and MeOH (100 ml), and the mixture was stirred at −40°C for 10 minutes. Then 13-[4-(*N*-methansulfonyl-*N*-methylamino)phenylethyloxy]-5-oxomilbemycin (10.8 g, 14.06 mmol) and boron trifluoride ethyl ether complex (0.06 ml) were added and the mixture was stirred at −45°C for 35 minutes. The reaction mixture was diluted with acetone (20 ml), warmed to 0°C, then EtOAc was added. The solution was washed with H_2O three times, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was crystallized from EtOAc and hexane to obtain **1** (8.20 g) as crystals. Then the filtrate was chromatographed on silica gel with the eluent (EtOAc : hexane=2 : 1) to retrieve additional **1** (0.887 g) from the filtrate. Thus, the total amount of **1** was 9.09 g (84.0% yield).

13-(4-Aminophenylethyloxy)-5-hydroxymilbemycin (**4**)

(1) 13-(4-Nitrophenyletyloxy)-5-oxomilbemycin

4-Nitrophenethyl alcohol (4.35 g, 26.0 mmol) was dissolved in 1,2-dichloroethane (25 ml), copper(I) iodide (1.05 g, 5.51 mmol), trifluoromethanesulfonic acid (0.77 ml), and a solution of 15-hydroxy-5-oxomilbemycin (5.35 mmol) in 1,2-dichloroethane (5 ml) were added. The mixture was stirred at room temperature for 25 minutes. The reaction mixture was diluted with EtOAc and washed with H_2O, 4% $NaHCO_3$, and H_2O again, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane=1 : 3) to obtain 13-(4-nitrophenyletyloxy)-5-oxomilbemycin (3.12 g, 82.5% yield).

(2) 13-(4-Aminophenylethyloxy)-5-hydroxymilbemycin (**4**)

13-(4-Nitrophenyletyloxy)-5-oxomilbemycin (1.685 g, 2.43 mmol) was dissolved in MeOH (33 ml) and cooled to 4°C, and then sodium borohydride (91 mg, 2.4 mmol) was added. The mixture was stirred at 4°C for 20 minutes. The reaction mixture was diluted with EtOAc, washed with H_2O twice, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was dissolved in AcOH (15 ml) and zinc dust (1.5 g) was added. The mixture was stirred at room temperature for 20 minutes in a water bath. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with H_2O three times, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain **4** (1.39 g, 86.2% yield).

13-(3,4-Dimethoxyphenyletyloxy)-5-hydroxymilbemycin (5)

(1) 13-(3,4-Dimethoxyphenyletyloxy)-5-oxomilbemycin

13-Iodomilbemycin (333 mg, 0.5 mmol) was dissolved in 1,2-dichloroethane (2.5 ml), 3,4-dimethoxyphenetyl alcohol (911.0 mg, 5.0 mmol) and silver oxide (1.0 g) were added. The mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc, washed with 10% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane = 1 : 4) to obtain 13-(3,4-dimethoxyphenyletyloxy)-5-oxomilbemycin (113.3 mg, 31.4% yield).

(2) 13-(3,4-Dimethoxyphenyletyloxy)-5-hydroxymilbemycin (**5**)

13-(3,4-Dimethoxyphenetyloxy)-5-oxomilbemycin (113.3 mg, 0.157 mmol) was dissolved in MeOH (3.7 ml) and the solution was cooled to 4°C, sodium borohydride (6.3 mg) was added. The mixture was stirred at 4°C for 30 minutes. The reaction mixture was diluted with EtOAc, washed with H_2O twice, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane = 35 : 65) to obtain **5** (69.0 mg, 60.8% yield): ^{1}H NMR ($CDCl_3$) δ 6.79 (1H, d, J=8.8 Hz, Ph-H), 6.75 (1H, s, Ph-H), 6.73 (1H, d, J=8.8 Hz, Ph-H), 5.70~5.84 (2H, m, C_9-H and C_{10}-H), 5.40 (1H, s, C_3-H), 5.17 (1H, m, C_{15}-H), 4.58 and 4.78 (2H, AB-q, J=15 Hz, C_{27}-CH_2), 4.29 (1H, d, J=6.2 Hz, C_5-H), 3.98 (1H, br-s, C_7-OH), 3.96 (1H, d, J=6.2 Hz, C_6-H), 3.87 and 3.86 (6H, two-s, OCH_3), 3.58 (1H, m, C_{17}-H), 3.53 and 3.30 (2H, m, C_{13}-OCH_2), 3.22 (1H, d, J=9.5 Hz, C_{13}-H), 3.05 (1H, m, C_{25}-H), 2.79 (2H, m, PhCH_2), 1.87 (3H, s, C_4-CH_3), 1.51 (3H, s, C_{14}-CH_3), 1.07 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.2 Hz, C_{24}-CH_3).

13-[4-(Acetylamino)phenylethyloxy]-5-hydroxymilbemycin (6)

Derivative **4** (304 mg, 0.45 mmol) was dissolved in dichloromethane (2.0 ml), acetic anhydride (0.051 mmol) and pyridine (44 μl, 0.54 mmol) were added. The mixture was stirred at room temperature for 20 minutes. EtOAc was added to the reaction mixture and the solution was washed with 0.5 M citric acid, H_2O, 4% $NaHCO_3$, H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane = 1 : 1) to obtain **6** (280 mg, 86.7% yield): MS m/z=719 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.40 (2H, d, J=8.4 Hz, Ph-H), 7.15 (2H, d, J=8.4 Hz, Ph-H), 7.10 (1H, br-s, NH), 5.40 (1H, s, C_3-H), 5.16 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-CH_2), 4.30 (1H, m, C_5-H), 3.99 (1H, s, C_7-OH), 3.95 (1H, d, J=6.3 Hz, C_6-H), 3.28 (1H, m, C_2-H), 3.20 (1H, d, J=9.9 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.80 (2H, m, PhCH_2), 2.17 (3H, s, CH_3CO), 1.87 (3H, s, C_4-CH_3), 1.04 (3H, d, J=6.0 Hz, C_{12}-CH_3), 0.98 (3H, dt, J=7.7 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.2 Hz, C_{24}-CH_3).

13-(2-Methoxy-2-phenylethyloxy)-5-hydroxymilbemycin (7)

Derivative **7** was prepared from 13-iodomilbemycin and 2-methoxy-2-phenylethanol in a similar manner as that described for the preparation of **5**: MS m/z=692 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.2~7.4 (5H, m, Ph-H), 5.40 (1H, s, C_3-H), 5.17 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-CH_2), 3.99 (1H, s, C_7-OH), 3.96 (1H, d, J=6.2 Hz, C_6-H), 3.55 (1H, m, C_{17}-H), 3.33 (1H, d, J=9.9 Hz, C_{13}-H), 3.28 (3H, s, OCH_3), 3.03 (1H, m, C_{25}-H), 1.87 (3H, s, C_4-CH_3), 1.10 (3H, d, J=6.2 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C25-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-(2-Hydroxy-2-phenylethyloxy)-5-hydroxymilbemycin (8)

(1) 13-[2-Phenyl-2-(tetrahydro-pyran-2-yloxy)ethyloxy]-5-oxomilbemycin

13-Iodomilbemycin (500 mg, 0.75 mmol) was dissolved in 1,2-dichloroethane (2.5 ml), 2-phenyl-2-(tetrahydropyran-2-yloxy)ethanol (836 mg, 3.75 mmol), 2,6-lutidine (0.09 ml, 0.78 mmol), and mercury(II) iodide (511 mg, 1.125 mmol) were added. The mixture was stirred at room temperature for 90 minutes. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with 20% sodium iodide twice, 10% $NaHCO_3$, 0.5 M citric acid, and H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : hexane = 1 : 4) to obtain 13-[2-phenyl-2-(tetrahydro-pyran-2-yloxy)-ethoxy]-5-oxomilbemycin (349 mg, 52.9% yield).

(2) 13-(2-Hydroxy-2-phenylethyloxy)-5-hydroxymilbemycin (8)

13-[2-Phenyl-2-(tetrahydropyran-2-yloxy)-ethyloxy]-5-oxomilbemycin (345 mg, 0.392 mmol) was dissolved in MeOH (10 ml), *p*-toluenesulfonic acid monohydrate (74.5 mg, 0.392 mmol) was added. The mixture was stirred at room temperature for 17 minutes. The reaction mixture was diluted with EtOAc, washed with 4% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : hexane = 4 : 6) to obtain 13-(2-hydroxy-

2-phenylethyloxy)-5-oxomilbemycin. A part of the residue (178 mg, 0.234 mmol) was dissolved in MeOH (7.0 ml), cooled to 4°C, and then sodiumborohydride (9.4 mg) was added. The mixture was stirred at 4°C for 15 minutes. The reaction mixture was diluted with EtOAc, washed with H_2O twice, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on ODS, with the eluent (80% acetonitrile) to obtain **8** (173 mg, quantitative): MS *m/z*=660 (M−H_2O); ^{1}H NMR ($CDCl_3$) δ 7.25~7.40 (5H, m, Ph-H), 5.40 (1H, s, C_3-H), 5.18 (1H, m, C_{15}-H), 4.88 (0.5H, dd, *J*=4.0 and 8.0 Hz, PhC*H*), 4.83 (0.5H, dd, *J*=3.3 and 8.8 Hz, PhC*H*), 4.69 (2H, s, C_{27}-CH_2), 4.29 (1H, m, C_5-H), 4.00 (1H, br-s, C_7-OH), 3.96 (1H, d, *J*=6.2 Hz, C_6-H), 3.56 (1H, m, C_{17}-H), 3.48 (1H, dd, *J*=3.3 and 9.9 Hz, C_{13}-OCH_2), 3.25~3.43 (2H, m, C_2-H and C_{13}-OCH_2), 3.24 (0.5H, d, *J*=9.9 Hz, C_{13}-H), 3.17 (0.5H, d, *J*=9.9 Hz, C_{13}-H), 3.04 (1H, m, C_{25}-H), 1.87 (3H, s, C_4-CH_3), 1.13 (1.5H, d, *J*=6.6 Hz, C_{12}-CH_3), 1.12 (1.5H, d, *J*=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, *J*=7.4 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, *J*=6.2 Hz, C_{24}-CH_3).

13-{5-[(*N*-Methylcarbamoyl)-amino]indan-2-yloxy}-5-hydroxymilbemycin (**9**) and (**10**)

(1) 13-(5-Nitro-2-indanyloxy)-5-oxomilbemycin

13-Iodomilbemycin (620 mg, 0.93 mmol) was dissolved in 1,2-dichloroethane (10 ml), 5-nitro-indan-2-ol (895 mg, 5.0 mmol) and mercury(II) iodide (650 mg, 1.43 mmol) were added. The mixture was stirred at 35°C for 90 minutes. Then 2,6-lutidine (0.12 ml) was added and the mixture was stirred at 35°C for 1 hour. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with 20% potassium iodide twice, 10% $NaHCO_3$, 0.5 N-HCl, H_2O, 4% $NaHCO_3$ and H_2O again, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane=1 : 3) to obtain 13-(5-nitro-2-indanyloxy) 5-oxomilbemycin (350 mg, 52.5% yield).

(2) 13-(5-Amino-2-indanyloxy)-5-hydroxymilbemycin

13-(5-Nitro-2-indanyloxy)-5-oxomilbemycin (350 mg, 0.487 mmol) was dissolved in MeOH (6.5 ml), the solution was cooled to 4°C. Sodiumborohydride (18 mg, 0.476 mmol) was added and the mixture was stirred at 4°C for 15 minutes. The reaction mixture was diluted with EtOAc, washed with H_2O twice, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was dissolved in 90% AcOH (3.5 ml) and zinc dust (350 mg) was added to the solution and the mixture was stirred for 20 minutes in a water bath. The reaction mixture was diluted with EtOAc, washed with H_2O three times, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain 13(5-amino-2-indanyloxy)-5-hydroxymilbemycin (261 mg, 77.8% yield).

(3) 13-{5-[(*N*-Methylcarbamoyl)-amino]indan-2-yloxy}-5-hydroxymilbemycin (**9**) and (**10**)

13-(5-Amino-2-indanyloxy)-5-hydroxymilbemycin (130 mg, 0.189 mmol) was dissolved in 1,2-dichloroethane (1.5 ml), methyl isocyanate (0.189 mmol) was added. The mixture was stirred at room temperature for 90 minutes. Then the reaction mixture was evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain **9** (24 mg): MS *m/z*=715 (M−CH_3NH_2); ^{1}H NMR ($CDCl_3$) δ 6.90~7.20 (3H, m, Ph-H), 6.19 (1H, br-s, NH), 5.41 (1H, s, C_3-H), 5.24 (1H, m, C_{15}-H), 4.70~4.67 (2H, AB-q, *J*=14.7 Hz, C_{27}-CH_2), 4.01 (1H, s, C_7-OH), 3.96 (1H, d, *J*=6.2 Hz, C_6-H), 3.60 (1H, m, C_{17}-H), 3.36 (1H, d, *J*=9.9 Hz, C_{13}-H), 3.27 (1H, m, C_2-H), 2.82 (3H, d, *J*=4.8 Hz, N-CH_3), 1.88 (3H, s, C_4-CH_3), 1.03, (3H, d, *J*=6.3 Hz, C_{12}-CH_3), 1.00 (3H, t, *J*=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, *J*=6.6 Hz, C_{24}-CH_3); and **10** (25 mg) MS *m/z*=715 ((M−CH_3NH_2)); ^{1}H NMR ($CDCl_3$) δ 6.90~7.20 (3H, m, Ph-H), 6.19 (1H, br-s, NH), 5.41 (1H, s, C_3-H), 5.24 (1H, m, C_{15}-H), 4.70~4.67 (2H, AB-q, *J*=14.7 Hz, C_{27}-CH_2), 4.01 (1H, s, C_7-OH), 3.96 (1H, d, *J*=6.2 Hz, C_6-H), 3.60 (1H, m, C_{17}-H), 3.36 (1H, d, *J*=9.9 Hz, C_{13}-H), 3.27 (1H, m, C_2-H), 2.82 (3H, d, *J*=4.8 Hz, N-CH_3), 1.88 (3H, s, C_4-CH_3), 1.02 (3H, d, *J*=6.3 Hz, C_{12}-CH_3), 1.00 (3H, t, *J*=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, *J*=6.6 Hz, C_{24}-CH_3).

13-[2-(4-*N*-Methylcarbamoylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**11**)

Derivative **11** was prepared from **4** in a similar manner as that described for the preparation of **9**-(3).

13-(4-Aminobenzyloxy)-5-hydroxymilbemycin (**12**)

(1) 13-(4-Nitrobenzyloxy)-5-oxomilbemycin

4-Nitrobenzyl alcohol (2.30 g, 15.0 mmol), mercury(II) iodide (2.06 g, 4.53 mmol) and 2,6-lutidine (0.36 ml, 3.09 mmol) were added to a solution of 13-iodomilbemycin (2.0 g, 3.0 mmol)[10] in 1,2-dichloroethane (10 ml). The mixture was stirred at room temperature for 90 minutes. The reaction mixture was diluted with EtOAc and washed with 20% KI twice, 10% $Na_2S_2O_3$, 0.5 N-HCl, H_2O, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on silica gel with the eluent (EtOAc : dichloromethane=15 : 85) to obtain 13-(4-nitrobenzyloxy)-5-oxomilbemycin (1.918 g, 92.4% yield).

(2) 13-(4-Nitrobenzyloxy)-5-hydroxymilbemycin

13-(4-Nitrobenzyloxy)-5-oxomilbemycin (1.918 g,

2.77 mmol) was dissolved in MeOH (37 ml) and cooled to 4°C, and then sodium borohydride (91 mg, 2.4 mmol) was added. The mixture was stirred at 4°C for 15 minutes. The reaction mixture was diluted with EtOAc, washed with H_2O twice, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (85% acetonitrile) to obtain 13-(4-nitrobenzyloxy)-5-hydroxymilbemycin (1.432 g, 74.6% yield).

(3) 13-(4-Aminobenzyloxy)-5-hydroxymilbemycin (**12**)

13-(4-Nitrobenzyloxy)-5-hydroxymilbemycin (1.2 g, 1.73 mmol) was dissolved in 90% AcOH (12 ml), zinc dust (1.2 g) was added. The mixture was stirred at room temperature for 20 minutes in a water bath. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with H_2O four times, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain **12** (1.092 g, 94.9% yield): MS *m/z*=663 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.09 (2H, d, J=8.4 Hz, Ph-H), 6.60 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, s, C_3-H), 4.68 (2H, s, C_{27}-H), 4.34 and 4.06 (2H, AB-q, J=11.4 Hz, $PhCH_2$), 4.28 (1H, m, C_5-H), 3.96 (1H, s, C_7-OH), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.65 (2H, br-s, NH_2), 3.60 (1H, m, C_{17}-H), 3.30 (1H, d, J=9.8 Hz, C_{13}-H), 3.27 (1H, m, C_2-H), 3.09 (1H, ddd, J=2.6, 8.8, and 8.8 Hz, C_{25}-H), 1.87 (3H, s, C_4-CH_3), 1.06 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.99 (3H, t, J=7.1 Hz, C_{25}-CH_2CH_3), 0.84 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[3-(4-Aminophenyl)propyloxy]-5-hydroxymilbemycin (**13**)

Derivative **13** was prepared from 15-hydroxy-5-oxomilbemycin and 3-(4-nitrophenyl)-propylalcohol in a similar manner as that described for the preparation of **4**. ^{1}H NMR ($CDCl_3$) δ 3.96 (1H, d, J=6.3 Hz, C_6-H), 3.19 (1H, d, J=9.7 Hz, C_{13}-H), 1.87 (3H, s, C_4-CH_3).

13-(3,4-Dimethoxybenzyloxy)-5-hydroxymilbemycin (**14**)

Derivative **14** was prepared from 13-iodomilbemycin and 3,4-dimethoxybenzyl alcohol in a similar manner as that described for the preparation of **5**: MS *m/z*=708 (M^+); ^{1}H NMR ($CDCl_3$) δ 6.86 (2H, d, J=10.0 Hz, Ph-H), 6.83 (1H, s, Ph-H), 5.40 (1H, s, C_3-H), 4.69 (2H, s, C_{27}-CH_2), 4.40 and 4.13 (2H, AB-q, J=11.4 Hz, $PhCH_2$), 3.95 (1H, d, J=6.3 Hz, C_6-H), 3.70 (6H, s, OCH_3), 3.59 (1H, m, C_{17}-H), 3.33 (1H, d, J=9.9 Hz, C_{13}-H), 3.26 (1H, m, C_2-H), 3.09 (1H, m, C_{25}-H), 1.83 (3H, s, C_4-CH_3), 1.10 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.99 (3H, t, J=7.0 Hz, C_{25}-CH_2CH_3).

13-[3-(3,4-Dimethoxyphenyl)propyloxy]-5-hydroxymilbemycin (**15**)

Derivative **15** was prepared from 13-iodomilbemycin and 3-(3,4-dimethoxyphenyl)propyl alcohol in a similar manner as that described for the preparation of **14**: MS *m/z*=736 (M^+); ^{1}H NMR ($CDCl_3$) δ 6.70~6.83 (3H, m, Ph-H), 5.41 (1H, s, C_3-H), 4.69 (2H, s, C_{27}-CH_2), 4.41 (1H, m, C_5-H), 4.03 (1H, d, J=6.2 Hz, C_6-H), 3.87 and 3.86 (6H, two-s, OCH_3), 3.58 (1H, m, C_{17}-H), 3.28 (1H, m, C_2-H), 3.20 (1H, d, J=9.8 Hz, C_{13}-H), 1.88 (3H, s, C_4-CH_3), 1.13 (3H, d, J=6.6 Hz, C_{12}-H), 0.99 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.83 (3H, d, J=6.2 Hz, C_{24}-CH_3).

13-[4-(Acetylamino)benzyloxy]-5-hydroxymilbemycin (**16**)

Derivative **16** was prepared from **12** in a similar manner as that described for the preparation of **6**: ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, d, J=6.5 Hz, C-24 CH_3), 0.99 (3H, t, J=7.3 Hz, C-25 CH_2CH_3), 1.08 (3H, d, J=6.4 Hz, C-12 CH_3), 1.87 (3H, s, C-4 CH_3), 2.18 (3H, s, acetyl H), 3.09 (1H, m, C-25 H), 3.58 (1H, m, C-17 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, d, J=5.9 Hz, C-5 H), 4.67 and 4.70 (2H, ABq, J=14.5 Hz, C-27 H), 7.31 (2H, d, J=8.2 Hz, Ph-H), 7.47 (2H, d, J=8.2 Hz, Ph-H).

13-[3-(4-*N*-Acetylaminophenyl)propyloxy]-5-hydroxymilbemycin (**17**)

The derivative **17** was prepared from **13** in a similar manner as that described for the preparation of **6**.

13-(2-Phenylethyloxy)-5-hydroxymilbemycin (**18**)

Derivative **18** was prepared from 13-iodomilbemycin and phenethyl alcohol in a similar manner as that described for the preparation of **5**: MS *m/z*=662 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.20~7.35 (5H, m, Ph-H), 5.70~5.85 (2H, m, C_9-H and C_{10}-H), 5.40 (1H, s, C_3-H), 4.68 (2H, s, C_{27}-CH_2), 4.30 (1H, m, C_5-H), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.22 (1H, d, J=10.0 Hz, C_{13}-H), 2.87 (2H, m, $PhCH_2$), 1.87 (3H, s, C_4-CH_3), 1.04 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.2 Hz, C_{24}-CH_3).

13-(1-Methyl-2-phenylethyloxy)-5-hydroxymilbemycin (**19**)

Derivative **19** was prepared from 13-iodomilbemycin and 1-methyl-2-phenylethanol in a similar manner as that described for the preparation of **5**: MS *m/z*=676 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.10~7.40 (5H, m, Ph-H), 5.40 (1H, m, C_3-H), 4.68 (1H, s, C_{27}-CH_2), 4.66 (1H, s, C_{27}-CH_2), 4.28 (1H, m, C_5-H), 3.95 (0.5H, d, J=6.2 Hz, C_6-H), 3.94 (0.5H,

d, J=6.2 Hz, C_6-H), 3.34 (0.5H, d, J=9.9 Hz, C_{13}-H), 3.28 (0.5H, d, J=9.9 Hz, C_{13}-H), 3.10 (0.5H, m, C_{25}-H), 3.06 (0.5H, m, C_{25}-H), 1.87 (3H, s, C_4-CH_3), 0.84 (1.5H, d, J=6.2 Hz, C_{24}-CH_3), 0.82 (1.5H, d, J=6.2 Hz, C_{24}-CH_3).

13-(2-Methyl-2-phenylethoxy)-5-hydroxymilbemycin (**20**)

Derivative **20** was prepared from 13-iodomilbemycin and 2-methyl-2-phenylethanol in a similar manner as that described for the preparation of **5**: MS m/z=676 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.15~7.35 (5H, m, Ph-H), 5.40 (1H, m, C_3-H), 5.14 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-CH_2), 4.29 (1H, m, C_5-H), 3.99 (1H, s, C_7-OH), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.57 (1H, m, C_{17}-H), 3.26 (1H, m, C_2-H), 3.20 (1H, d, J=9.7 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 1.87 (3H, s, C_4-CH_3), 1.28 (3H, d, J=6.6 Hz, PhCHCH_3), 1.05 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2-(2-Aminophenyl)ethyloxy]-5-hydroxymilbemycin (**21**)

Derivative **21** was prepared from 15-hydroxy-5-oxomilbemycin and 2-nitrophenethyl alcohol in a similar manner as that described for the preparation of **4**: MS m/z=677 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.0~7.1 (2H, m, Ph-H), 6.7~6.8 (2H, m, Ph-H), 5.7~5.8 (2H, m, C_9 and C_{10}-H), 5.39 (1H, s, C_3-H), 5.17 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-H), 4.29 (1H, m, C_5-H) 3.98 (1H, br-s, C_7-OH), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.23 (1H, d, J=9.9 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.79 (2H, t, J=6.2 Hz, $PhCH_2$), 1.87 (3H, s, C_4-CH_3), 1.05 (3H, d, J=6.2 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.5 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2-(3-Aminophenyl)ethyloxy]-5-hydroxymilbemycin (**22**)

Derivative **22** was prepared from 15-hydroxy-5-oxomilbemycin and 3-nitrophenethyl alcohol in a similar manner as that described for the preparation of **4**: MS m/z=677 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.14 (1H, m, Ph-H), 6.7~6.8 (3H, m, Ph-H), 5.40 (1H, s, C_3-H), 5.17 (1H, m, C_{15}-H), 4.69 and 4.66 (2H, AB-q, J=14.6 Hz, C_{27}-H), 4.29 (1H, m, C_5-H), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.21 (1H, d, J=9.9 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.79 (2H, m, $PhCH_2$), 1,87 (3H, s, C_4-CH_3), 1.05 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.4 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2-(2-*N*-Methylcarbamoylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**23**)

The derivative **23** was prepared from **21** in a similar manner as that described for the preparation of **9**-(**3**): MS m/z=703 ($M-CH_3NH_2$); ^{1}H NMR ($CDCl_3$) δ 7.0~7.5 (4H, m, Ph-H), 5.40 (1H, s, C_3-H), 5.15 (1H, m, C_{15}-H), 4.67 (2H, s, C_{27}-H), 4.29 (1H, m, C_5-H), 3.96 (1H, s, C_7-OH), 3.95 (1H, d, J=5.9 Hz, C_6-H), 3.18 (1H, d, J=9.9 Hz, C_{13}-H), 3.05 (1H, m, C_{25}-H), 2.84 (3H, d, J=5.1 Hz, NCH_3), 1.83 (3H, s, C_4-CH_3), 1.02 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.97 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2(3-*N*-Methylcarbamoylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**24**)

Derivative **24** was prepared from **22** in a similar manner as that described for the preparation of **9**-(**3**): ^{1}H NMR ($CDCl_3$) δ 7.22 (1H, m, Ph-H), 7.17 (1H, s, Ph-H), 7.07 (1H, d, J=8.1 Hz, Ph-H), 6.96 (1H, d, J=7.3 Hz, Ph-H), 6.25 (1H, s, NH), 5.40 (1H, s, C_3-H), 5.16 (1H, m, C_{15}-H), 4.29 (1H, m, C_5-H), 4.00 (1H, s, C_7-OH), 3.96 (1H, d, J=5.8 Hz, C_6-H), 3.21 (1H, d, J=9.6 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.83 (3H, d, J=4.8 Hz, NCH_3), 1.87 (3H, s, C_4-CH_3), 1.03 (3H, d, J=6.6 Hz, C_{12}-H), 0.98 (3H, t, J=7.5 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2-(4-Methoxycarbonylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**25**)

Derivative **25** was prepared from **4** and methyl chloroformate in a similar manner as that described for the preparation of **6**: MS m/z=764 (M^+); ^{1}H NMR ($CDCl_3$) δ 8.80 (1H, s, NH), 7.54 (2H, d, J=8.8 Hz, Ph-H), 7.22 (2H, d, J=8.8 Hz, Ph-H), 5.40 (1H, s, C_3-H), 5.16 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-H), 4.29 (1H, m, C_5-H), 3.70 (3H, s, OCH_3), 3.20 (1H, d, J=9.9 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.83 (2H, m, $PhCH_2$), 1.87 (3H, s, C_4-CH_3), 1.03 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.2 Hz, C_{24}-CH_3).

13-[2-(4-Methanesulfonylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**26**)

Derivative **26** was prepared from **4** and methanesulfonyl chloride in a similar manner as that described for the preparation of **6**.

13-[2-(4-Cyanoacetylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**27**)

Cyanoacetic acid (42.5 mg, 0.50 mmol) was dissolved in 1,2-dichloroethane (2.5 ml) and the solution was cooled to 4°C. Then pyridine (0.05 ml), 2-chloroformyl-1,2,4-

triazolo[4,3-*a*]pyridin-3-one[12] (100 mg, 0.50 mmol) and **4** (203 mg, 0.30 mmol) were added. The mixture was stirred at room temperature for 90 minutes then at 35°C for an extra hour. The reaction mixture was diluted with EtOAc, washed with 1 N-HCl, H_2O, 4% $NaHCO_3$, and H_2O again, dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain crude **27**. The crude was chromatographed on silica gel with the eluent (EtOAc : cyclohexane=3 : 1) to obtain pure **27** (120 mg, 53.7% yield): ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, s, NH), 7.40 (2H, d, J=8.4 Hz, Ph-H), 7.20 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, s, C_3-H), 5.17 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-H), 4.29 (1H, m, C_5-H), 3.98 (1H, s, C_7-OH), 3.95 (1H, d, J=5.2 Hz, C_6-H), 3.54 (2H, s, $NCCH_2$), 3.20 (1H, d, J=9.9 Hz, C_{13}-H), 3.06 (1H, m, C_{25}-H), 2.82 (2H, m, $PhCH_2$), 1.87 (3H, s, C_4-CH_3), 1.03 (3H, d, J=6.6 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d, J=6.6 Hz, C_{24}-CH_3).

13-[2-(4-Methoxyacetylaminophenyl)ethyloxy]-5-hydroxymilbemycin (**28**)

Derivative **28** was prepared from **4** and methoxyacetyl chloride in a similar manner as that described for the preparation of **6**: MS m/z=749 (M^+); ^{1}H NMR ($CDCl_3$) δ 8.19 (1H, s, NH), 7.47 (2H, d, J=8.4 Hz, Ph-H), 7.17 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, m, C_{15}-H), 4.68 (2H, s, C_{27}-H), 4.29 (1H, m, C_5-H), 4.01 (2H, s, $MeOCH_2$), 4.00 (1H, s, C_7-OH), 3.95 (1H, d, J=6.2 Hz, C_6-H), 3.50 (3H, s, CH_3O), 3.21 (1H, d, J=9.9 Hz, C_{13}-H), 3.07 (1H, m, C_{25}-H), 2.81 (2H, m, $PhCH_2$), 1.87 (3H, s, C_4-CH_3), 1.04 (3H, d, J=6.2 Hz, C_{12}-CH_3), 0.98 (3H, t, J=7.3 Hz, C_{25}-CH_2CH_3), 0.82 (3H, d J=6.6 Hz, C_{24}-CH_3).

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