CHEMICAL MODIFICATION OF TYLOSIN: SYNTHESIS OF AMINO DERIVATIVES AT C-20 POSITION OF TYLOSIN AND DEMYCAROSYLTYLOSIN

HAJIME MATSUBARA, JUNJI INOKOSHI, AKIRA NAKAGAWA, HARUO TANAKA and SATOSHI ŌMURA*

School of Pharmaceutical Sciences, Kitasato University and The Kitasato Institute, Minato-ku, Tokyo 108, Japan

(Received for publication July 14, 1983)

Reductive aminations of the aldehyde group at C-20 position of tylosin and demycarosyltylosin (desmycosin) were carried out using primary and secondary amines in the presence of sodium cyanoborohydride. Some of these derivatives brought about higher antimicrobial and ribosome-binding activities, and the structure-activity relationship is discussed.

Tylosin, a 16-membered macrolide antibiotic, has strong antimicrobial activity against Gram-positive bacteria and mycoplasmas, and has been widely used as a feed additive and as a therapeutic agent in the treatment of mycoplasmosis (*Mycoplasma gallisepticum*) in the veterinary field. The chemical modification of 16-membered macrolide antibiotics such as leucomycin, midecamycin, spiramycin and maridomycin was investigated to focus chiefly on acylation on the hydroxyl groups of the aglycone and sugar moieties[1]. Among these derivatives, the acyl derivatives of C-3″ and/or C-4″ position were superior in antimicrobial activity and blood levels *in vivo*[2,3]. Many acyl derivatives of tylosin have been also reported[4~6], but the modification of the aldehyde group at C-20 position or the carbonyl group at C-9 position in tylosin has not been undertaken. Recently, we reported the antimicrobial and ribosome-binding activities of tylosin-related compounds[7,8]. On the other hand, erythromycylamine synthesized from erythromycin by MASSEY *et al.*[9] showed enhanced antimicrobial activity against several test organisms. We have previously reported the synthesis of the primary amino derivative at C-20 and the dimeric compound of tylosin, and showed that tylosin dimer reveals a similar level of antimicrobial activity and ribosome-binding activity to those of tylosin[10].

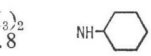
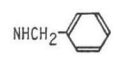
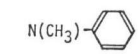
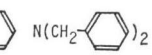
We attempted the synthesis of the substituted amino derivatives at C-20, which were prepared by reductive amination of tylosin or demycarosyltylosin with primary or secondary amines and sodium cyanoborohydride. In this paper, we describe the synthesis of new amino derivatives of tylosin and demycarosyltylosin and the relationship between their structures and antimicrobial and ribosome-binding activities.

Synthesis of 20-Amino Derivatives

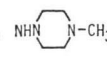
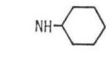
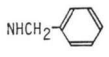
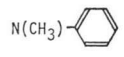
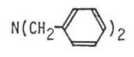
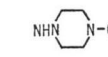
In our previous paper[10], we reported the syntheses of the primary amino derivative and the dimeric compound of tylosin by reductive amination using ammonium acetate. The additional substituted amino derivatives of tylosin at C-20 were prepared as follows. A mixture of tylosin (**1**), amine (10 equiv) and sodium cyanoborohydride (4 equiv) in methanol was stirred at room temperature in a nitrogen atmosphere. After completion of the reaction, the reaction product was purified by silica gel column chromatography to obtain a pure powder of a 20-amino derivative of tylosin. In a similar manner,

* To whom all correspondence should be addressed.

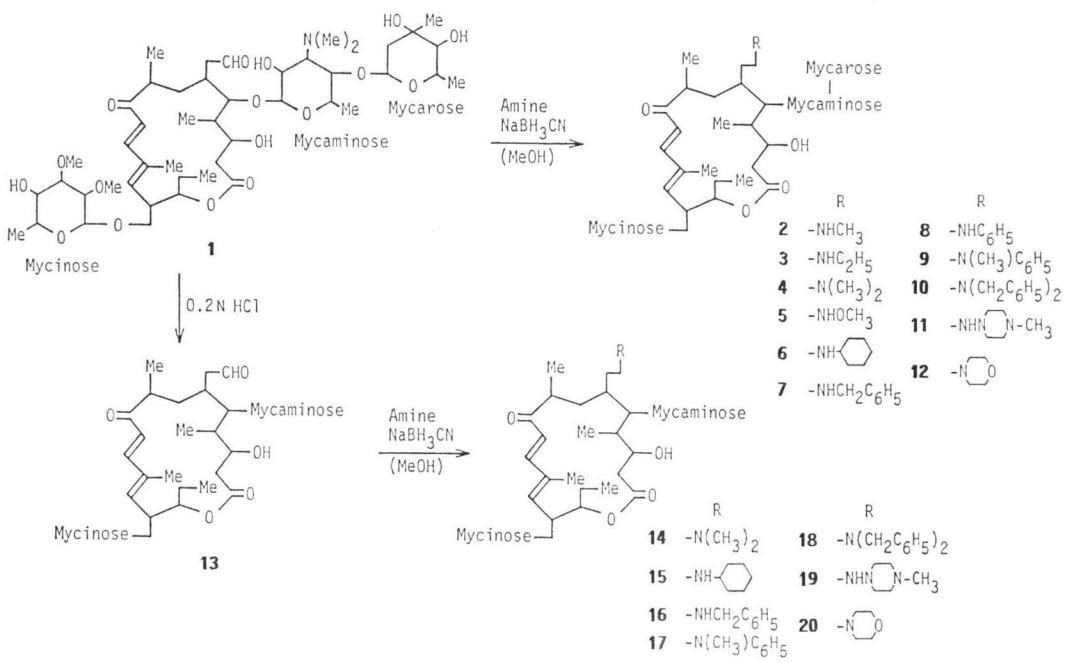
Table 1. ^{13}C NMR assignments for 20-amino-20-deoxo

Carbon	1	2	4	6	7	9	10
Aglycone							
1	174.4	173.9	173.2	173.9	173.9	174.9	174.9
2	39.4	39.6	39.6	39.4	39.5	38.3	39.6
3	71.8	71.9	72.0	72.0	72.0	72.0	71.9
4	45.1	45.1	45.3	45.1	45.0	45.0	45.1
5	81.6	80.2	80.2	82.0	82.1	82.1	82.1
6	32.9	35.1	33.0	33.4	34.0	34.0	34.0
7	32.2	33.8	34.3	32.7	33.1	33.8	33.1
8	40.3	39.6	39.6	41.1	41.1	39.8	41.0
9	203.6	204.6	204.5	204.7	204.5	204.5	203.9
10	118.9	118.8	118.4	118.4	118.6	118.4	118.6
11	148.5	148.6	148.4	148.5	148.5	147.8	147.9
12	135.2	135.0	134.8	135.1	135.2	135.5	135.3
13	142.6	143.3	143.5	143.4	142.9	141.9	142.0
14	44.8	45.1	45.3	45.1	45.0	45.0	45.1
15	74.9	75.1	74.7	75.1	75.1	75.2	75.2
16	25.5	25.5	25.5	25.5	25.5	25.5	25.5
17	9.0	9.5	9.2	9.5	9.4	9.5	9.8
18	9.7	9.6	9.7	9.6	9.6	9.7	9.8
19	43.9	33.8	34.3	31.6	34.0	34.3	34.1
20	203.4	48.7	56.7	45.1	48.2	51.5	58.5
21	13.0	12.9	12.9	13.0	13.0	13.0	13.0
22	17.4	17.8	17.7	17.7	17.6	17.5	17.7
23	69.7	69.7	69.7	69.7	69.7	69.7	69.7
Mycaminose							
1	104.0	104.3	104.4	104.4	104.3	104.7	104.6
2	69.1	69.1	69.0	69.0	69.0	69.1	69.0
3	69.1	69.1	69.4	69.3	69.3	69.4	69.0
4	75.4	75.1	75.3	75.4	75.3	75.6	75.7
5	73.3	73.0	73.2	73.2	73.2	73.4	73.0
6	18.3	18.3	18.3	18.3	19.1	18.3	18.3
7	42.1	42.1	42.1	42.1	42.2	42.2	42.1
8	42.1	42.1	42.1	42.1	42.2	42.2	42.1
Mycarose							
1	96.6	96.7	96.7	96.7	96.7	96.8	96.7
2	41.0	41.1	41.1	41.1	41.1	41.1	41.1
3	69.7	69.7	69.7	69.7	69.7	69.7	69.7
4	76.6	76.6	76.6	76.6	76.7	76.7	76.6
5	66.3	66.1	66.2	66.2	66.2	66.2	66.3
6	19.1	19.2	19.2	19.3	19.1	19.4	19.1
7	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Mycinose							
1	101.3	101.3	101.4	101.4	101.4	101.3	101.3
2	82.1	82.0	82.0	82.1	82.1	82.1	82.1
3	80.0	80.1	80.1	80.1	80.1	80.1	80.1
4	72.9	73.0	72.9	73.0	73.0	72.9	73.0
5	70.7	70.6	70.7	70.7	70.7	70.7	70.7
6	17.8	17.8	17.8	17.8	17.8	17.8	17.8
7	59.8	59.7	59.8	59.8	59.8	59.8	59.8
8	61.9	61.9	61.9	61.9	61.9	61.8	61.9
		$NHCH_3$ 42.1	$N(CH_3)_2$ 44.8				
				1 : 56.1 2,6: 32.5 3,5: 25.1 4 : 25.8	1 : 139.1 2,3, 5,6: 128.9 4 : 127.6 α : 53.0	1 : 150.0 2,6: 112.9 3,5: 129.5 4 : 116.4 CH_3: 42.2	1 : 139.5 2,6: 128.7 3,5: 128.5 4 : 127.3 α : 53.2

derivatives of tylosin and demycarosyltylosin.

11	12	14	15	16	17	18	19	20
174.3	173.2	173.4	173.9	173.8	174.9	174.9	174.2	173.3
39.7	39.6	39.6	39.6	39.6	38.3	39.5	39.7	39.6
71.9	72.0	71.3	71.0	71.0	71.2	71.0	71.3	71.1
45.1	45.3	45.2	45.1	45.1	45.0	45.1	45.0	45.3
82.0	82.1	82.0	82.0	82.1	82.1	82.1	82.0	82.1
34.0	32.3	34.3	33.9	34.1	34.0	34.3	33.7	33.9
34.0	32.3	34.3	33.9	34.1	34.0	34.3	32.9	33.9
39.7	39.6	39.6	39.6	39.6	39.8	40.8	39.7	39.6
204.2	203.8	204.7	204.8	204.6	204.5	203.3	204.4	204.8
118.8	118.6	118.6	118.7	118.6	118.0	117.8	118.5	118.6
148.1	148.2	148.5	148.6	148.1	147.1	147.6	148.2	148.3
135.2	134.8	138.8	135.1	135.1	135.5	135.2	135.2	134.9
142.3	143.5	143.4	143.1	142.7	141.7	142.0	142.6	143.5
45.1	45.3	45.2	45.1	45.1	45.0	45.1	45.0	46.5
75.2	74.7	74.9	75.1	75.0	75.6	75.2	75.2	74.6
25.5	25.5	25.4	25.5	25.4	25.6	25.6	25.5	25.5
9.5	9.3	9.1	9.3	9.6	9.7	9.0	9.0	9.2
9.6	9.6	9.7	9.6	9.6	9.7	9.7	9.6	9.6
33.8	32.3	34.1	31.7	34.1	33.8	34.3	33.7	33.9
51.6	55.0	56.7	45.1	45.8	51.5	58.5	51.6	55.1
12.9	13.0	12.9	13.0	13.0	13.0	13.0	12.9	13.0
17.8	17.8	17.8	17.8	17.8	17.8	17.8	17.8	17.8
69.7	69.7	70.6	70.7	70.6	70.5	70.9	70.6	70.4
104.4	104.2	104.7	104.6	104.5	105.0	104.1	104.8	104.5
69.2	69.0	69.3	69.3	69.3	69.4	69.3	69.3	69.4
69.2	69.3	69.3	69.3	69.3	69.4	69.3	69.3	69.4
75.3	75.4	70.6	70.7	70.7	70.5	70.9	70.6	70.4
73.0	73.2	73.5	73.6	73.4	73.7	73.2	73.4	73.4
18.3	18.3	18.0	18.0	17.8	18.2	17.8	17.8	17.8
42.1	42.1	41.9	41.9	41.8	41.9	41.8	41.8	41.9
42.1	42.1	41.9	41.9	41.8	41.9	41.8	41.8	41.9
96.7	96.7							
41.1	41.1							
69.7	69.7							
76.6	76.6							
66.1	66.3							
19.2	19.2							
25.5	25.5							
101.3	101.4	101.3	101.4	101.3	101.3	101.3	101.3	101.4
82.0	82.1	82.0	82.0	82.1	82.1	82.1	82.0	82.1
80.1	80.1	80.2	80.1	80.1	80.0	80.1	80.1	80.1
73.0	72.9	73.0	73.4	73.0	72.9	72.9	72.9	72.9
70.6	70.7	70.6	70.7	70.7	70.8	70.9	70.6	70.7
17.8	17.8	17.8	17.8	17.8	17.8	17.8	17.8	17.8
59.8	59.8	59.7	59.8	59.8	59.8	59.8	59.7	59.8
61.9	61.9	61.8	61.9	61.8	61.9	61.9	61.9	61.8
		$N(CH_3)_2$ 44.6						
CH_2: 54.5 54.9 CH_3: 45.1	1: 66.6 2: 53.7		1 : 56.0 2,6: 32.6 3,5: 25.1 4 : 25.9	1 : 140.0 2,3, 5,6: 128.7 4 : 127.3 α : 53.3	1 : 150.0 2,6: 112.8 3,5: 129.5 4 : 116.3 CH_3: 41.9	1 : 139.4 2,6: 128.4 3,5: 129.7 4 : 127.0 α : 55.6	CH_2: 54.5 54.8 CH_3: 45.9	1: 66.6 2: 53.7

Scheme 1.



reductive amination of demycarosyltylosin (**13**) obtained by mild acid hydrolysis of tylosin afforded the corresponding 20-amino derivatives. Various primary and secondary amines (methylamine, ethylamine, dimethylamine, methoxyamine, cyclohexylamine, 1-amino-4-methylpiperazine, morpholine, benzylamine, aniline, dibenzylamine and *N*-methylaniline) were used for the reductive amination. The 20-amino derivatives obtained by the above reductive amination of tylosin and demycarosyltylosin were shown in Scheme 1. The structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR (Table 1) and mass spectral data.

Biological Activities and Ribosome-binding Activities

The minimal inhibitory concentrations (MIC, μg/ml) of the amino derivatives against a variety of bacteria were assayed by the agar dilution method. The MIC values are shown in Table 2.

Among the tylosin derivatives, 20-(*N*-benzylamino)-20-deoxotylosin (**7**), 20-anilino-20-deoxotylosin (**8**) and 20-deoxo-20-morpholinotylosin (**12**) showed an increase of antimicrobial activity against some bacteria, but other derivatives showed a slight decrease in antimicrobial activity.

Among demycarosyltylosin derivatives, 20-deoxo-20-(*N*-methylanilino)demycarosyltylosin (**17**) was more active against Gram-positive bacteria than compound **13**. However, the other derivatives of **13** had similar or less activities. Interestingly, 20-deoxo-20-(di-*N*-methylamino)demycarosyltylosin (**14**) and 20-(*N*-benzylamino)-20-deoxodemycarosyltylosin (**16**) showed enhanced activity against the acid-fast bacterium *Mycobacterium smegmatis* KB 42 (ATCC 607).

In addition, we measured the ribosome-binding affinities (ID_{50}, μM: 50% inhibition dose of [10, 11,12,13-^{3}H]tetrahydroleucomycin A_8 binding to *Escherichia coli* ribosomes) of these amino derivatives. The ID_{50} values are available for the evaluation of *in vitro* activity. In fact, in the acyl derivatives at C-4″ of leucomycin a definitive relationship between the ID_{50} values and MIC was indicated[11]. The

Table 2. Antimicrobial activities and affinities to ribosomes of 20-amino-20-deoxo derivatives of tylosin and demycarosyltylosin.

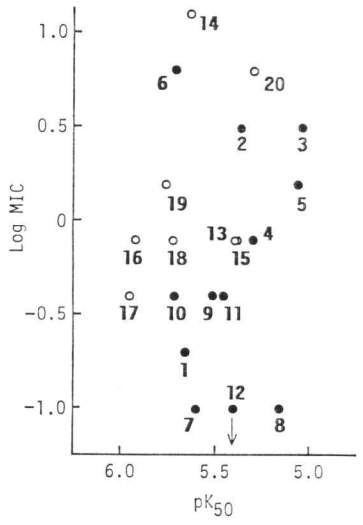
Concentration (ID_{50}) of a compound for 50% inhibition of [^{3}H]tetrahydroleucomycin A_8 binding to ribosomes and MIC were assayed as described in the text.

Compound	MIC (μg/ml)*							ID_{50} (μM)
	SA	BS	BC	ML	MS	EC	KP	
1	0.78	0.2	0.4	<0.1	100	100	25	2.2
2	25	3.12	3.12	1.56	>100	>100	>100	4.9
3	50	3.12	3.12	0.78	>100	>100	>100	9.1
4	25	0.78	1.56	0.4	>100	>100	>100	4.9
5	6.25	1.56	1.56	0.4	>100	>100	100	8.5
6	12.5	6.25	0.78	0.4	>100	>100	100	1.9
7	6.25	0.1	0.4	<0.1	50	>100	>100	2.5
8	1.56	0.1	0.2	<0.1	100	>100	50	6.9
9	3.12	0.4	0.4	0.1	>100	>100	>100	3.0
10	3.12	0.4	0.78	<0.1	50	100	50	1.9
11	1.56	0.4	0.4	0.2	>100	>100	50	3.5
12	0.78	<0.1	0.4	<0.1	>100	50	25	3.9
13	1.56	0.78	0.4	<0.1	>100	100	25	4.1
14	12.5	12.5	3.12	0.4	25	>100	>100	2.3
15	3.12	0.78	0.78	<0.1	>100	100	100	4.2
16	1.56	0.78	0.4	<0.1	3.12	100	50	1.2
17	0.2	0.4	<0.1	<0.1	100	>100	25	1.1
18	1.56	0.78	0.78	<0.1	100	>100	100	1.9
19	3.12	1.56	1.56	0.2	>100	>100	>100	1.7
20	12.5	6.25	1.56	0.2	>100	>100	50	4.9

* Test organism. SA, *Staphylococcus aureus* KB 210 (ATCC 6538P); BS, *Bacillus subtilis* KB 211 (ATCC 6633); BC, *B. cereus* KB 143 (IFO 3001); ML, *Micrococcus luteus* KB 212 (ATCC 9341); MS, *Mycobacterium smegmatis* KB 42 (ATCC 607); EC, *Escherichia coli* KB 212 (ATCC 9341); KP, *Klebsiella pneumoniae* KB 13 (PCI 602).

Fig. 1. Log MIC as a function of pK_{50} of [^{3}H]-tetrahydroleucomycin A_8 binding to ribosomes for 20-amino-20-deoxo derivatives of tylosin (●) and demycarosyltylosin (○).

The MIC values against *B. subtilis* (Table 2) was used. The pK_{50} is the negative log of ID_{50} (Table 2).



ID_{50} values for the amino derivatives of **1** and **13** are shown in Table 2. Further the correlation between pK_{50} and log (MIC against *Bacillus subtilis*) is shown in Fig. 1.

As shown in Fig. 1, a definitive relationship between the ID_{50} values and MIC was not observed. Since the variation of the amino group in tylosin derivatives did not greatly affect their

Table 3. *In vivo* antimicrobial activities of the 20-amino-20-deoxo derivatives against *Streptococcus pyogenes* C 203 infection in mice and their *in vitro* activities.

Compound	ED_{50} (mg/kg)		MIC (μg/ml)
	Subcutaneous	Oral	
1	0.5	33	0.25
10	>10	76	0.5
13	0.8	76	0.25
16	<1.5	39	0.25
18	6.6	35	0.25
20	2.3	>100	0.5

affinities to the ribosomes, the variation of the derivatives in antimicrobial activity seems to be due to their variation in permeability to the cells. Fig. 1 also suggests that the high antimicrobial activities of **7**, **8** and **12** are due to their high permeabilities to the cells. Among demycarosyltylosin derivatives, most compounds except **17** did not show higher antimicrobial activities, though they had higher affinities to ribosomes than demycarosyltylosin (**13**). The amino derivatives of **13** except the *N*-cyclohexylamino and *N*-methylanilino derivatives (**15** and **17**) had similar levels of ribosome affinities to the corresponding tylosin derivatives, but had lower antimicrobial activities. This suggests that the demycarosyltylosin derivatives are less permeable to the cells than the tylosin derivatives.

The *in vivo* antimicrobial activities of some derivatives (**10**, **16**, **18** and **20**) were assayed in mice infected with *Streptococcus pyogenes* C 203. The results are shown in Table 3. The *in vivo* activities of compounds **16** and **18** from oral administration were higher than that of **13** and reached that of **1**, but the other compounds were less active.

Although the modifications of the aldehyde group such as the reduction to alcohol and reductive amination resulted in a decrease of antimicrobial activity, the introduction of the amines bearing aromatic moieties to the aldehyde group brought about higher antimicrobial activities.

Experimental

The optical rotations were measured on a Jasco DIP-181 spectrometer. The UV spectra were measured on a Shimadzu UV-210A spectrometer. Mass spectra were taken with a Jeol JMS-D 100 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian EM-390 90 MHz spectrometer and a Jeol PS 100 spectrometer, respectively, with tetramethylsilane as an internal standard in $CDCl_3$. Column chromatography was performed on Merck silica gel 60. For analytical TLC plates Merck silica gel 60 F_{254} was used (solvent 1; $CHCl_3$ - MeOH - conc. ammonia water, 10: 1: 0.05, solvent 2; $CHCl_3$ - MeOH - conc. ammonia water, 6: 1: 0.05).

MIC against various bacteria was assayed by the agar dilution method using a medium containing 0.5% peptone and 0.5% meat extract (pH 7.0). Concentration (ID_{50}) of a compound for 50% inhibition of [10,11,12,13-^{3}H]tetrahydroleucomycin A_3 binding to ribosomes for *E. coli* was determined as described previously[12].

General Method of Reductive Amination

A mixture of tylosin (or demycarosyltylosin), amine (10 equiv) and sodium cyanoborohydride (4 equiv) in methanol was stirred at room temperature in a nitrogen atmosphere. When the reaction was completed, the mixture was poured into cold aqueous sodium bicarbonate, and the product was extracted with chloroform from aqueous solution. The chloroform layer was dried over anhydrous sodium sulfate and then evaporated to dryness under reduced pressure. The crude product was subjected to silica gel column chromatography to give a reductive aminated product.

20-Deoxo-20-(*N*-methylamino)tylosin (**2**)

Yield 47%: TLC Rf 0.22 (solvent 1): $[\alpha]_D^{29}$ −51.1° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (19,000): Mass (*m/z*) 755 (M^+ −mycinose), 596 (aglycone+mycaminose), 175 (mycinose), 157 (mycaminose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.39 (3H, s, NHCH_3 at C-20 position), 2.47 (6H, s, H-7′ and H-8′), 3.44, 3.57 (each 3H, s, H-7‴ and H-8‴), 4.25 (1H, d, *J*=7.0 Hz, H-1′), 4.54 (1H, d, *J*=7.5 Hz, H-1‴), 4.95 (1H, bt, $J_{15,16}$=9.0 Hz, H-15), 5.06 (1H, d, *J*=2.5 Hz, H-1″), 5.91 (1H, bd, *J*=11.0 Hz, H-13), 6.25 (1H, d, *J*=15.0 Hz, H-10), 7.34 (1H, d, *J*=15.0 Hz, H-11).

20-Deoxo-20-(*N*-ethylamino)tylosin (**3**)

Yield 41%: TLC Rf 0.45 (solvent 1): $[\alpha]_D^{29}$ −49.3° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (19,400): Mass (*m/z*) 928 ($M^+ - H_2O$), 783 (M^+ −mycarose), 610 (aglycone+mycinose), 191, 175 (mycinose): ^{1}H NMR δ (ppm) 1.79 (3H, s, H-22), 2.49 (6H, s, H-7′ and H-8′), 3.46, 3.60 (each 3H, s, H-7‴ and H-8‴), 4.26 (1H, d, *J*=7.0 Hz, H-1′), 4.55 (1H, d, *J*=8.0 Hz, H-1‴), 4.95 (1H, bt, $J_{15,16}$=15.0 Hz, H-15),

5.06 (1H, d, J=2.5 Hz, H-1″), 5.91 (1H, d, J=10.5 Hz, H-13), 6.27 (1H, d, J=15.0 Hz, H-10), 7.34 (1H, d, J=15.0 Hz, H-11).

20-Deoxo-20-(di-*N*-methylamino)tylosin (**4**)

Yield 82%: TLC Rf 0.38 (solvent 1): $[\alpha]_D^{29}$ −40.4° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 nm (12,500): Mass (*m/z*) 944 (M^+), 800 (M^+ −mycarose), 770 (M^+ −mycinose), 419 (aglycone), 175 (mycinose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.15 (6H, s, $N(CH_3)_2$ at C-20 position), 2.52 (6H, s, H-7′ and H-8′), 3.43, 3.60 (each 3H, s, H-7‴ and H-8‴), 4.23 (1H, d, J=8.4 Hz, H-1′), 4.52 (1H, d, J=9.0 Hz, H-1‴), 4.92 (1H, b, H-15), 5.05 (1H, d, J=3.0 Hz, H-1″), 5.90 (1H, d, J=10.5 Hz, H-13), 6.26 (1H, d, J=15.0 Hz, H-10), 7.33 (1H, d, J=15.0 Hz, H-11).

20-Deoxo-20-(*N*-methoxyamino)tylosin (**5**)

Yield 74%: TLC Rf 0.49 (solvent 1): $[\alpha]_D^{29}$ −80.4° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 278 (14,600): Mass (*m/z*) 946 (M^+), 771 (M^+ −mycinose), 421 (aglycone), 175 (mycinose), 157 (mycaminose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.47 (6H, s, H-7′ and H-8′), 3.50, 3.60 (each 3H, s, H-7‴ and H-8‴), 3.88 (3H, s, $NHOCH_3$), 4.54 (1H, d, J=8.0 Hz, H-1‴), *ca.* 5.0 (b, H-15), 5.07 (1H, d, J=3.0 Hz, H-1″), 5.55 (b, H-13), 6.83 (1H, d, J=15.0 Hz, H-10), 6.93 (1H, d, J=15.0 Hz, H-11).

20-(*N*-Cyclohexylamino)-20-deoxotylosin (**6**)

Yield 50%: TLC Rf 0.49 (solvent 1): $[\alpha]_D^{29}$ −48.9° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (20,000): Mass (*m/z*) 832 (M^+ −mycarose), 664 (aglycone+mycinose), 473 (aglycone), 175 (mycinose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.47 (6H, s, H-7′ and H-8′), 3.47, 3.60 (each 3H, s, H-7‴ and H-8‴), 4.26 (1H, d, J=7.2 Hz, H-1′), 4.53 (1H, d, J=8.7 Hz, H-1‴), 4.95 (1H, bt, H-15), 5.06 (1H, d, J=3.0 Hz, H-1″), 5.88 (1H, d, J=10.0 Hz, H-13), 6.23 (1H, d, J=15.5 Hz, H-10), 7.30 (1H, d, J=15.0 Hz, H-11).

20-(*N*-Benzylamino)-20-deoxotylosin (**7**)

Yield 70%: TLC Rf 0.45 (solvent 1): $[\alpha]_D^{29}$ −55.8° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (22,900): Mass (*m/z*) 1,006 (M^+), 846 (M^+ −mycarose), 672 (aglycone+mycinose), 654 (aglycone+mycaminose), 175 (mycinose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.47 (6H, s, H-7′ and H-8′), 3.47, 3.58 (each 3H, s, H-7‴ and H-8‴), 4.20 (1H, d, J=6.9 Hz, H-1′), 4.55 (1H, d, J=7.8 Hz, H-1‴), 4.92 (1H, b, H-15), 5.07 (1H, d, J=3.5 Hz, H-1″), 5.87 (1H, d, J=9.6 Hz, H-13), 6.24 (1H, d, J=16.5 Hz, H-10), *ca.* 7.3 (H-11 and aromatic protons).

20-Anilino-20-deoxotylosin (**8**)

Yield 49%: TLC Rf 0.28 (solvent 1): $[\alpha]_D^{29}$ −86.0° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 250 (15,600), 282 (21,600): Mass (*m/z*) 831 (M^+ −mycarose), 658 (aglycone+mycinose), 190 (mycaminose), 175 (mycinose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.46 (6H, s, H-7′ and H-8′), 3.46, 3.56 (each 3H, s, H-7‴ and H-8‴), 4.23 (1H, d, J=7.5 Hz, H-1′), 4.54 (1H, d, J=7.5 Hz, H-1‴), 4.97 (1H, bt, H-15), 5.04 (1H, d, J=3.0 Hz, H-1″), 5.84 (1H, bd, J=10.5 Hz, H-13), 6.23 (1H, d, J=15.5 Hz, H-10), 6.57 (2H, d, J=7.0 Hz, H-2 and H-6 in aromatic ring), 6.67 (1H, d, J=7.0 Hz, H-4 in aromatic ring), 7.10 (2H, d, J=7.0 Hz, H-3 and H-5 in aromatic ring), 7.23 (1H, d, J=15.5 Hz, H-11).

20-Deoxo-20-(*N*-methylanilino)tylosin (**9**)

Yield 34%: TLC Rf 0.41 (solvent 1): $[\alpha]_D^{29}$ −84.3° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 260 (20,000), 282 (20,900): Mass (*m/z*) 1,006 (M^+), 862 (M^+ −mycarose), 688 (aglycone+mycinose), 481 (aglycone), 175 (mycinose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.79 (3H, s, H-22), 2.47 (6H, s, H-7′ and H-8′), 2.87 (3H, s, NCH_3 at C-20 position), 3.46, 3.59 (each 3H, s, H-7‴ and H-8‴), 4.27 (1H, d, J=7.5 Hz, H-1′), 4.54 (1H, d, J=7.5 Hz, H-1‴), *ca.* 5.0 (b, H-15), 5.05 (1H, d, J=3.0 Hz, H-1″), 5.83 (1H, bd, J=11.0 Hz, H-13), 6.21 (1H, d, J=15.0 Hz, H-10), 6.6～6.8 (H-2, H-4 and H-6 in aromatic ring), 7.1～7.2 (H-3 and H-5 in aromatic ring, H-11).

20-Deoxo-20-(di-*N*-benzylamino)tylosin (**10**)

Yield 70%: TLC Rf 0.57 (solvent 1): $[\alpha]_D^{29}$ −51.3° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (23,000): Mass (*m/z*) 1,096 (M^+), 936 (M^+ −mycarose), 762 (aglycone+mycinose), 744 (aglycone+mycaminose), 175 (mycinose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.46 (6H, s, H-7′ and H-8′), 3.50, 3.60 (each 3H,

s, H-7‴ and H-8‴), 4.57 (1H, d, J=8.0 Hz, H-1‴), 5.88 (1H, bd, J=10.5 Hz, H-13), 6.23 (1H, d, J=15.0 Hz, H-10), *ca.* 7.3 (H-11 and aromatic protons).

20-Deoxo-20-[*N*-(4-methylpiperazinyl)amino]tylosin (**11**)

Yield 69%: TLC Rf 0.26 (solvent 1): $[\alpha]_D^{29}$ −55.4° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (17,700): Mass (*m/z*) 1,014 (M^+), 870 (M^+ −mycarose), 680 (aglycone+mycinose), 175 (mycinose), 145 (mycarose): ^{1}H NMR δ (ppm) 1.79 (3H, s, H-22), 2.31 (3H, s, ), 2.47 (6H, s, H-7′ and H-8′), 3.47, 3.60 (each 3H, s, H-7‴ and H-8‴), 4.24 (1H, d, J=7.0 Hz, H-1′), 4.56 (1H, d, J=7.5 Hz, H-1‴), 4.95 (b, H-15), 5.07 (1H, d, J=3.0 Hz, H-1″), 5.86 (1H, d, J=10.5 Hz, H-13), 6.26 (1H, d, J=15.0 Hz, H-10), 7.27 (1H, d, J=15.0 Hz, H-11).

20-Deoxo-20-morpholinotylosin (**12**)

Yield 79%: TLC Rf 0.55 (solvent 1): $[\alpha]_D^{31}$ −39.6° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (19,500): Mass (*m/z*) 668, 666 (aglycone+mycinose), 650 (aglycone+mycaminose), 191 (mycinose), 174 (mycaminose), 86 (morpholine): ^{1}H NMR δ (ppm) 1.80 (3H, s, H-22), 2.49 (6H, s, H-7′ and H-8′), 3.47, 3.60 (each 3H, s, H-7‴ and H-8‴), 4.23 (1H, d, J=7.5 Hz, H-1′), 4.56 (1H, d, J=8.0 Hz, H-1‴), 4.96 (1H, bt, $J_{15,16}$=9.0 Hz, H-15), 5.06 (1H, d, J=3.0 Hz, H-1″), 5.96 (1H, bd, J=10.5 Hz, H-13), 6.28 (1H, d, J=15.0 Hz, H-10), 7.25 (1H, d, J=15.0 Hz, H-11).

20-Deoxo-20-(di-*N*-methylamino)demycarosyltylosin (**14**)

Yield 74%: TLC Rf 0.58 (solvent 2): $[\alpha]_D^{29}$ −14.2° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (17,000): Mass (*m/z*) 800 (M^+), 610 (aglycone+mycinose), 419 (aglycone), 191 (mycinose), 174 (mycaminose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.22 (6H, s, $N(CH_3)_2$ at C-20 position), 2.50 (6H, s, H-7′ and H-8′), 3.43, 3.60 (each 3H, s, H-7″ and H-8″), 4.27 (1H, d, J=7.5 Hz, H-1′), 4.55 (1H, d, J=7.5 Hz, H-1″), 4.9 (b, H-15), 5.90 (1H, d, J=10.5 Hz, H-13), 6.26 (1H, d, J=15.0 Hz, H-10), 7.32 (1H, d, J=15.0 Hz, H-11).

20-(*N*-Cyclohexylamino)-20-deoxodemycarosyltylosin (**15**)

Yield 41%: TLC Rf 0.60 (solvent 2): $[\alpha]_D^{29}$ −21.9° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (22,400): Mass (*m/z*) 854 (M^+), 664 (aglycone+mycinose), 473 (aglycone), 175 (mycinose), 174 (mycaminose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.50 (6H, s, H-7′ and H-8′), 3.46, 3.60 (each 3H, s, H-7″ and H-8″), 4.30 (1H, d, J=8.0 Hz, H-1′), 4.56 (1H, d, J=8.0 Hz, H-1″), 4.86 (1H, bt, $J_{15,16}$=9.0 Hz, H-15), 5 88 (1H, bd, J=10.5 Hz, H-13), 6.27 (1H, d, J=15.0 Hz, H-10), 7.33 (1H, d, J=15.0 Hz, H-11).

20-(*N*-Benzylamino)-20-deoxodemycarosyltylosin (**16**)

Yield 61%: TLC Rf 0.62 (solvent 2): $[\alpha]_D^{29}$ −35.2° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 284 (22,000): Mass (*m/z*) 862 (M^+), 510 (aglycone+mycaminose), 191 (mycinose), 106 (benzylamine): ^{1}H NMR δ (ppm) 1.80 (3H, s, H-22), 2.43 (6H, s, H-7′ and H-8′), 3.48, 3.60 (each 3H, s, H-7″ and H-8″), 4.23 (1H, d, J=8.0 Hz, H-1′), 4.55 (1H, d, J=8.0 Hz, H-1″), 5.4 (1H, bt, $J_{15,16}$=9.0 Hz, H-15), 5.87 (1H, d, J=10.5 Hz, H-13), 6.27 (1H, d, J=15.0 Hz, H-10), 7.25 (1H, d, J=15.0 Hz, H-11), 7.3 (aromatic protons).

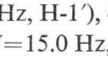
20-Deoxo-20-(*N*-methylanilino)demycarosyltylosin (**17**)

Yield 43%: TLC Rf 0.64 (solvent 2): $[\alpha]_D^{29}$ −42.1° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (20,600): Mass (*m/z*) 862 (M^+), 688 (M^+ −mycaminose), 190, 174 (mycaminose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.49 (6H, s, H-7′ and H-8′), 2.87 (3H, s, NCH_3 at C-20 position), 3.46, 3.59 (each 3H, s, H-7″ and H-8″), 4.33 (1H, d, J=7.5 Hz, H-1′), 4.56 (1H, d, J=8.0 Hz, H-1″), 5.00 (1H dt, $J_{14,15}$=2.0 Hz, $J_{15,16}$=8.0 Hz, H-15), 5.84 (1H, bd, J=11.0 Hz, H-13), 6.63 (1H, d, J=15.5 Hz, H-10), 6.6～6.8 (H-2, H-4 and H-6 in aromatic ring), 7.2～7.4 (H-3 and H-5 in aromatic ring and H-11).

20-Deoxo-20-(di-*N*-benzylamino)demycarosyltylosin (**18**)

Yield 62%: TLC Rf 0.70 (solvent 2): $[\alpha]_D^{29}$ −29.4° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (22,000): Mass (*m/z*) 952 (M^+), 762 (aglycone+mycinose), 175 (mycinose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.46 (6H, s, H-7′ and H-8′), 3.50, 3.60 (each 3H, s, H-7″ and H-8″), 4.54 (1H, d, J=8.0 Hz, H-1″), 5.06 (1H, bt, $J_{15,16}$=7.5 Hz, H-15), 5.90 (1H, bd, J=10.5 Hz, H-13), 6.25 (1H, d, J=15.0 Hz, H-10), 7.2～7.5 (H-11 and aromatic protons).

20-Deoxo-20-[*N*-(4-methylpiperazinyl)amino]demycarosyltylosin (**19**)

Yield 70%: TLC Rf 0.36 (solvent 2): $[\alpha]_D^{29}$ −29.2° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (23,100): Mass (*m/z*) 871, 680 (aglycone+mycinose), 522 (aglycone), 175 (mycinose), 174 (mycaminose): ^{1}H NMR δ (ppm) 1.77 (3H, s, H-22), 2.30 (3H, s, ), 2.47 (6H, s, H-7′ and H-8′), 3.46, 3.59 (each 3H, s, H-7″ and H-8″), 4.27 (1H, d, *J*=7.0 Hz, H-1′), 4.56 (1H, d, *J*=8.0 Hz, H-1″), 4.93 (bt, H-15), 5.84 (1H, bd, *J*=11.0 Hz, H-13), 6.25 (1H, d, *J*=15.0 Hz, H-10), 7.27 (1H, d, *J*=15.0 Hz, H-11).

20-Deoxo-20-morpholinodemycarosyltylosin (**20**)

Yield 44%: TLC Rf 0.76 (solvent 2): $[\alpha]_D^{31}$ −20.3° (*c* 1, MeOH): UV λ_{max}^{MeOH} nm (ε) 283 (19,500): Mass (*m/z*) 842 (M^+), 824 (M^+-H_2O), 669 (M^+−mycaminose), 477, 461 (aglycone), 174 (mycaminose): ^{1}H NMR δ (ppm) 1.78 (3H, s, H-22), 2.49 (6H, s, H-7′ and H-8′), 3.46, 3.59 (each 3H, s, H-7″ and H-8″), 4.26 (1H, d, *J*=7.0 Hz, H-1′), 4.56 (1H, d, *J*=8.0 Hz, H-1″), 4.94 (1H, bt, $J_{15,16}$=9.0 Hz, H-15), 5.84 (1H, bd, *J*=11.0 Hz, H-13), 6.27 (1H, d, *J*=15.0 Hz, H-10), 7.24 (1H, d, *J*=15.0 Hz, H-11).

Acknowledgments

The authors thank Dr. H. SANO for variable discussion. They also thank Mr. R. MASUMA for determination of antimicrobial activities, and Mr. H. KUSAYANAGI and Miss H. OGAWA for helpful technical assistance. They are grateful to Dr. H. A. KIRST, Microbiological and Fermentation Products Research, Eli Lilly Company, for his generous gift of tylosin.

References

1) ŌMURA, S. & H. SAKAKIBARA: Chemical modification of 16-membered macrolide antibiotics. Hakko to Kogyo 37: 1171~1186, 1979
2) SAKAKIBARA, H.; O. OKEKAWA, T. FUJIWARA, M. OTANI & S. ŌMURA: Acyl derivatives of 16-membered macrolides. I. Synthesis and biological properties of 3″-*O*-propionylleucomycin A_5 (TMS-19-Q). J. Antibiotics 34: 1001~1010, 1981
3) SAKAKIBARA, H.; O. OKEKAWA, T. FUJIWARA, M. AIZAWA & S. ŌMURA: Acyl derivatives of 16-membered macrolides. II. Antibacterial activities and serum levels of 3″-*O*-acyl derivatives of leucomycin. J. Antibiotics 34: 1011~1018, 1981
4) OKAMOTO, R.; H. NOMURA, M. TSUCHIYA, H. TSUNEKAWA, T. FUKUMOTO, T. INUI, T. SAWA, T. TAKEUCHI & H. UMEZAWA: The activity of 4″-acylated tylosin derivatives against macrolide-resistant Gram-positive bacteria. J. Antibiotics 32: 542~544, 1979
5) OKAMOTO, R.; T. FUKUMOTO, H. NOMURA, K. KIYOSHIMA, K. NAKAMURA, A. TAKAMATSU, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Physico-chemical properties of new acyl derivatives of tylosin produced by microbial transformation. J. Antibiotics 33: 1300~1308, 1980
6) OKAMOTO, R.; M. TSUCHIYA, H. NOMURA, H. IGUCHI, K. KIYOSHIMA, S. HORI, T. INUI, T. SAWA, T. TAKEUCHI & H. UMEZAWA: Biological properties of new acyl derivatives of tylosin. J. Antibiotics 33: 1309~1315, 1980
7) MATSUBARA, H.; K. MIYANO, A. NAKAGAWA & S. ŌMURA: Chemical transformation of tylosin, a 16-membered macrolide, and its structure-activity relationship. Chem. Pharm. Bull. 30: 97~110, 1982
8) ŌMURA, S.; J. INOKOSHI, H. MATSUBARA & H. TANAKA: Ribosome-binding activities and antimicrobial activities of tylosin and its related compounds. J. Antibiotics 36: 1709~1712, 1983
9) MASSEY, E. H.; B. KITCHELL, L. D. MARTIN, K. GERZON & H. W. MURPHY: Erythromycylamine. Tetrahedron Lett. 1970: 157~160, 1970
10) ŌMURA, S.; K. MIYANO, H. MATSUBARA & A. NAKAGAWA: Novel dimeric derivatives of leucomycins and tylosin, sixteen-membered macrolides. J. Med. Chem. 25: 271~275, 1982
11) ŌMURA, S.; A. NAKAGAWA, H. SAKAKIBARA, O. OKEKAWA, R. BRANDSH & S. PESTKA: Structure-activity relationship among the *O*-acyl derivatives of leucomycin. Correlation of minimal inhibitory concentrations with binding to *Escherichia coli* ribosome. J. Med. Chem. 20: 732~736, 1977
12) ŌMURA, S.; H. TANAKA, J. INOKOSHI, H. SAKAKIBARA & T. FUJIWARA: Binding of [^{3}H]tetrahydroleucomycin A_3 to *Escherichia coli* ribosomes and the effect of 3″-*O*-acyl derivatives of leucomycins on the binding. J. Antibiotics 35: 491~496, 1982