CHEMICAL MODIFICATION OF TYLOSIN

THIOETHER DERIVATIVES OF TYLOSIN AND DEMYCAROSYLTYLOSIN

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(Received for publication March 29, 1984)

Thioether derivatives of tylosin and demycarosyltylosin were synthesized by MICHAEL-type addition of thiol to C-11 of the enone moiety on the aglycone. Some of tylosin derivatives were effective to macrolide resistant *Staphylococcus aureus*, and their *in vivo* activities were same or superior than that of tylosin.

Tylosin (**1**), 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 treatment of mycoplasmosis caused by *Mycoplasma gallisepticum* in veterinary field. Chemical modifications of **1** have been described focusing chiefly on acylation and sulfonylation of the hydroxyl group at the C-4 position of mycarose.[1] In previous papers,[2,3] we have reported reduction of the formyl group at C-19 and/or hydroxymethyl group at C-14 of 5-*O*-mycaminosyltylonolide obtained by a vigorous acid hydrolysis of **1** into methyl group and the reductive amination of the formyl group. Among these derivatives, some compounds showed enhanced *in vivo* activity against *Streptococcus pyogenes*.

In this paper, we describe the synthesis of new thioether derivatives by MICHAEL-type addition of several thiols to the C-11 position and their evaluation for *in vitro* and mouse *in vivo* activities.

Synthesis of Thioether Derivatives by MICHAEL-type Addition

Application of the MICHAEL addition of thiophenol to C-11 of **1** in the presence of triethylamine afforded only the intramolecular aldol product between C-8 and C-20, but the desired 11-thioether derivative was not recognized in this condition (Chart 1). A similar aldol product has been also obtained by a vigorous acid hydrolysis of **1**.[2] The structure of **3** was assigned as 8,20-cyclo-20-hydroxytylosin based on the following spectral data; EI-mass: M^+ m/z 915; UV λ_{max}^{MeOH} 276 nm; ^{13}C NMR δ 73.7 (d, C-20), 36.8 (s, C-8). Therefore, for the preparation of thioether derivatives by MICHAEL-

Chart 1.

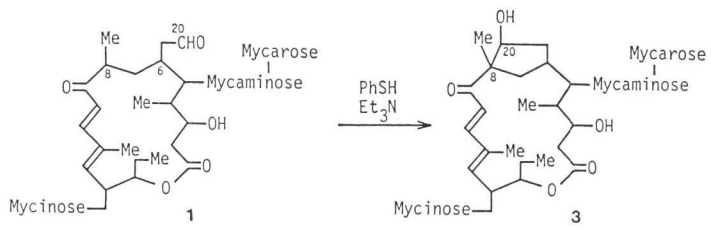


Table 1. ^{13}C NMR assignments for 11-thioether derivatives.

Carbon	1	16	18	22	24	25	19	20	21
Aglycone									
1	174.4	172.7	173.2	172.5	172.9	171.1	172.3	173.1	172.7
2	39.4	39.5	40.2	39.4	39.2	40.9	40.3	40.8	39.2
3	71.8	71.7	71.3	73.0	73.0	73.0	71.7	71.8	71.4
4	45.1	44.8	44.9	44.8	44.5	45.1	44.9	44.8	44.8
5	81.6	82.1	82.2	82.0	82.1	82.1	82.2	82.2	82.5
6	32.9	32.6	32.4	32.7	32.5	32.6	32.6	32.6	32.8
7	32.2	31.6	31.5	31.6	31.4	33.1	32.7	31.9	32.5
8	40.3	40.3	41.1	40.3	40.3	40.9	41.1	40.2	40.3
9	203.6	210.0	210.3	210.1	210.3	210.9	210.1	209.8	210.2
10	118.9	33.0	33.8	32.7	33.0	32.1	33.0	32.6	33.4
11	148.5	53.6	49.4	53.7	48.6	46.5	53.8	53.8	54.8
12	135.2	134.8	136.8	134.8	136.8	138.1	134.3	133.5	136.6
13	142.6	128.0	126.7	128.0	126.6	122.3	126.0	128.9	126.4
14	44.8	44.5	42.9	44.4	44.5	44.6	44.9	44.8	44.8
15	74.9	75.2	74.6	76.1	75.9	76.3	75.0	75.2	74.5
16	25.5	25.5	25.6	25.4	25.3	25.6	25.5	25.5	25.5
17	9.0	8.0	9.0	8.6	8.7	7.9	8.8	9.0	8.7
18	9.7	9.7	9.6	9.7	9.6	10.0	9.1	9.6	9.7
19	43.9	44.5	42.9	44.4	44.5	44.6	44.9	42.9	44.5
20	203.4	203.1	203.4	203.3	203.4	203.0	203.1	203.0	203.1
21	13.0	12.1	11.6	12.1	11.6	10.0	12.3	12.2	12.2
22	17.4	17.8	17.8	17.5	17.9	16.5	18.0	17.9	17.5
23	69.7	69.7	69.9	69.5	70.6	70.9	70.0	69.8	69.9
Mycaminose									
1	104.0	104.2	104.1	104.6	104.5	105.5	104.3	104.1	104.3
2	69.1	69.0	69.3	70.7	70.6	70.9	69.2	69.1	69.3
3	69.1	69.0	69.3	70.7	70.6	70.9	69.2	69.1	69.3
4	75.4	76.1	76.0	70.7	70.6	70.9	75.0	76.0	75.5
5	73.3	73.4	73.4	73.6	73.5	73.8	73.4	73.4	73.4
6	18.3	18.3	18.3	17.9	17.9	18.1	18.3	18.3	18.3
7	42.1	42.1	42.7	41.8	41.8	41.9	42.2	42.1	42.2
8	42.1	42.1	42.7	41.8	41.8	41.9	42.2	42.1	42.2

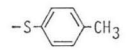
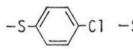
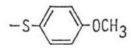
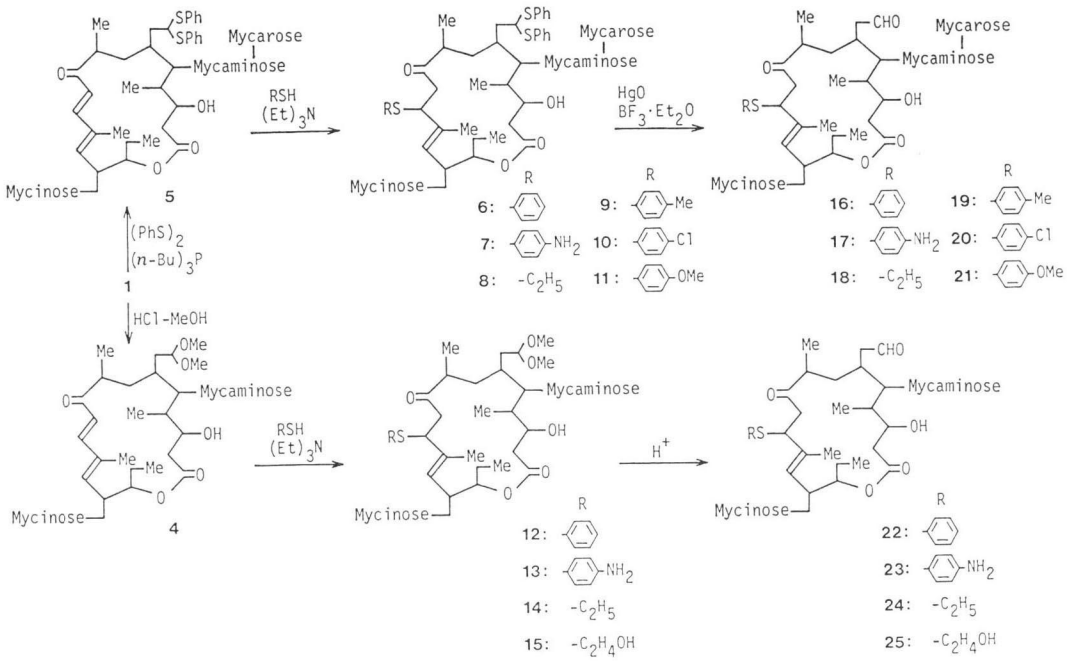
Mycarose									
1	96.6	96.8	96.5				96.7	96.5	96.5
2	41.0	41.1	41.1				41.1	41.1	41.1
3	69.7	69.7	69.9				70.0	69.8	69.9
4	76.6	76.4	76.4				76.4	76.7	76.7
5	66.3	66.3	66.6				66.5	66.4	66.6
6	19.1	19.1	19.6				19.6	19.1	19.6
7	25.5	25.5	25.6				25.5	25.5	25.5
Mycinose									
1	101.3	100.7	101.4	100.7	100.8	100.7	100.8	101.0	100.8
2	82.1	82.1	82.2	82.0	82.1	83.3	82.2	82.2	82.2
3	80.0	79.7	79.8	79.7	79.8	79.3	79.7	79.8	79.7
4	72.9	73.0	73.0	73.0	73.0	73.0	72.9	73.0	73.4
5	70.7	70.7	70.8	69.5	70.6	70.9	70.9	70.9	70.9
6	17.8	17.9	17.8	17.9	17.9	17.8	18.0	17.9	17.8
7	59.8	59.6	59.7	59.5	59.5	59.5	59.6	59.6	59.6
8	61.9	61.8	61.8	61.7	61.6	61.6	61.8	61.8	61.7
		–SPh	$-SCH_2CH_3$	–SPh	$-SCH_2CH_3$	$-SCH_2CH_2OH$			
		1 : 136.5 2, 6: 133.9 3, 5: 129.1 4 : 126.7	CH_2: 25.3 CH_3: 14.6	1 : 136.5 2, 6: 133.8 3, 5: 129.1 4 : 126.6	CH_2: 25.3 CH_3: 14.5	1: 36.6 2: 61.9	1 : 138.2 2, 6: 130.0 3, 5: 134.3 4 : 137.4 CH_3: 21.2	1 : 136.3 2, 6: 129.4 3, 5: 135.2 4 : 135.8	1, 2, 6: 136.6 3, 5 : 114.8 4 : 160.3 CH_3 : 55.5

Chart 2. Synthesis of 10,11-dihydro-11-thioether derivatives.



type addition, the formyl group at 19 position was protected. Protection of the aldehyde group of **1** with hydrogen chloride - methanol afforded demycarosyltylosin dimethyl acetal (**4**) with cleavage of the glycosidic linkage between mycaminose and mycarose. Protection of the formyl group with diphenyldisulfide and tri-*n*-butylphosphine in chloroform[4] afforded diphenyl dithioacetal (**5**) without liberation of mycarose moiety. MICHAEL-type addition of thiols such as thiophenol, 4-aminothiophenol, ethanethiol, 4-methylthiophenol, 4-chlorothiophenol, 4-methoxythiophenol and 2-mercaptoethanol in the presence of triethylamine to both acetal **4** and **5** afforded the corresponding thioethers **6**~**15**. Diphenyl dithioacetal compounds **6**~**11** were hydrolyzed with mercury oxide (red) and boron trifluoride etherate in 15% aqueous tetrahydrofuran under nitrogen atmosphere[5] to yield 10,11-dihydro-11-thioether derivatives (**16**~**21**). On the other hand, the hydrolysis of dimethyl acetal compounds **12**~**15** with 0.1 N HCl - acetonitrile gave demycarosyltylosin thioether derivatives **22**~**25** (Chart 2). The structural evidence for thioether formation were deduced from mainly NMR spectral data. In the ^{1}H NMR spectrum of **16** the signals of H-10 and H-11 observed at δ 6.26 and 7.33 in **1** appeared to shift to up field region and the signals of H-13 and the methyl at C-12 were observed to shift to δ 4.84 ($J_{13,14}$ = 9.5 Hz) and 1.63 from δ 5.91 and 1.76, respectively. The ^{13}C NMR signals of C-10 and C-11 observed at δ 118.9 and 148.5 in **1** appeared to shift to δ 33.0 and 53.6 in **16**, respectively, indicating that phenylthio group was introduced to 11 position. In a similar manner, the structures of other thioether derivatives were also confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral data. The ^{13}C chemical shift values for **1** and the thioether derivatives are shown in Table 1.

Biological Activities

Minimal inhibitory concentrations (MIC, μg/ml) of the thioether derivatives against some Gram-positive and negative bacteria are shown in Table 2. Generally, the thioether derivatives showed some-

Table 2. *In vitro* and *in vivo* antimicrobial activities.

		MIC (μg/ml)*							ED_{50} (mg/kg)**	
		SA	MC^r	BS	BC	ML	EC	KP	sc	po
Tylosin	**(1)**	0.78	>100	0.2	0.4	0.1	100	25	0.75	50
$-SC_6H_5$	**(16)**	1.56	50	0.4	0.4	<0.1	100	100	7.9	54
$-SC_6H_4NH_2$	**(17)**	6.25	>100	3.12	1.56	0.78	>100	>100		
-SEt	**(18)**	1.56	>100	0.78	0.78	<0.1	100	100		
$-SC_6H_4Me$	**(19)**	3.12	25	0.4	0.4	<0.1	100	100	5.0	33
$-SC_6H_4Cl$	**(20)**	3.12	25	0.4	0.4	<0.1	100	100	7.4	54
$-SC_6H_4OMe$	**(21)**	3.12	50	0.4	0.4	<0.1	100	100		
Demycarosyltylosin	**(2)**	0.4	>100	0.4	0.2	<0.1	50	12.5	0.8	
$-SC_6H_5$	**(22)**	0.78	>100	0.78	0.2	<0.1	100	100		
$-SC_6H_4NH_2$	**(23)**	3.12	>100	3.12	0.78	0.2	>100	>100		
-SEt	**(24)**	1.56	>100	0.78	0.78	0.2	>100	>100		
$-SCH_2CH_2OH$	**(25)**	12.5	>100	12.5	3.12	0.4	>100	>100		

* SA: *Staphylococcus aureus* KB210 (ATCC 6538P), MC^r: *S. aureus* KB199 (macrolide-resistant), BS: *Bacillus subtilis* KB211 (ATCC 6633), BC: *B. cereus* KB143 (IFO 3001), ML: *Micrococcus luteus* KB212 (ATCC 9341), EC: *Escherichia coli* KB213 (NIHJ), KP: *Klebsiella pneumoniae* KB13 (PCI 602).

** *Streptococcus pyogenes* C-203 infection in mice.

what decreased antimicrobial activity against Gram-positive and negative bacteria compared with **1** and **2**. However, compounds **16**, **19**, **20** and **21** in which phenylthio group has been introduced, exhibited enhanced activity against *Staphylococcus aureus* EM^r KB199, macrolide-resistant strain which shows resistance for tylosin at the concentration of 1,000 μg/ml. On the other hand, 11-*p*-aminophenylthio-10,11-dihydrotylosin (**17**), 10,11-dihydro-11-ethylthiotylosin (**18**) and thioether derivatives of demycarosyltylosin, **22**~**25** showed no antimicrobial activity against the resistant strain. These results indicate that introduction of an aromatic ring and presence of the mycarose moiety in thioether derivatives might be important factors contributing to the antimicrobial activity against the macrolide resistant strain. *In vivo* activity of **16**, **19** and **20** was tested in mice infected with *Streptococcus pyogenes* C-203 and the results are shown in Table 2. These derivatives were less active than **1** subcutaneously, but the ED_{50} values by oral administration were the same as or better than that of **1**.

As described above, thioether derivatives obtained by MICHAEL-type addition possessed the same antimicrobial activity against macrolide-sensitive strains as **1** and **2**. And the derivatives with the substituents such as methyl, methoxy and chlorophenylthio group showed enhanced antimicrobial activity against a macrolide-resistant strain. The results in our previous and present reports provide a new direction for chemical modification of tylosin and its related antibiotics.

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. ^1H and ^{13}C NMR spectra were obtained on a Varian EM-390 90 MHz spectrometer and a Jeol PS 100 spectrometer, with tetramethylsilane as an internal standard in $CDCl_3$. Column chromatography was performed on Merck silica gel 60. Merck silica gel 60 F_{254} was used for analytical TLC plates.

MIC against various bacteria was assayed by the agar dilution method using a medium containing peptone 0.5% and meat extract 0.5% (pH 7.0). *In vivo* test in mice infected *S. pyogenes* C-203 was carried out in the procedure by KIRST *et al.*[6]

Demycarosyltylosin Dimethyl Acetal (**4**)

Tylosin (10.0 g) was dissolved in 0.5% HCl - MeOH, and the solution was allowed to stand for 1 hour at room temperature. The reaction mixture was poured into cold sodium bicarbonate solution and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated *in vacuo* to give **4** (8.5 g, yield 95%). TLC Rf 0.70 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −4.7° (*c* 1.0, MeOH); EI-mass *m/z* 817 (M^+), 468 (aglycone), 190 and 174 (mycaminose); ^1H NMR δ 1.69 (3H s, H-22), 2.49 (6H s, $N(CH_3)_2$), 3.23, 3.28 (each 3H s, 20-$(OCH_3)_2$), 3.45 (3H s, 2″-OCH_3), 3.58 (3H s, 3″-OCH_3), 4.30 (1H d, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.55 (1H d, $J_{1'',2''}$ = 7.5 Hz, H-1″), 4.96 (1H bt, $J_{15,16}$ = 7.5 Hz, H-15), 5.86 (1H bd, $J_{13,14}$ = 11.0 Hz, H-13), 6.25 (1H d, $J_{10,11}$ = 15.0 Hz, H-10), 7.28 (1H d, $J_{10,11}$ = 15.0 Hz, H-11).

Tylosin Diphenyl Dithioacetal (**5**)

Tylosin (10.0 g), diphenyl disulfide (3.6 g) and tri-*n*-butylphosphine (4.5 ml) were dissolved in $CHCl_3$ (20 ml), and the mixture was stirred for 2 hours at room temperature under nitrogen atmosphere. The reaction mixture was concentrated *in vacuo*, and the residue was subjected to silica gel column chromatography ($CHCl_3$ - MeOH - conc NH_4OH, 50: 1: 0.05) to give compound **5** (7.9 g, yield 65%). TLC Rf 0.69 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −67.2° (*c* 1.0, MeOH); EI-mass *m/z* 1,117 (M^+), 608 (aglycone), 318 (mycarosylmycaminose), 191 (mycinose); ^1H NMR δ 1.77 (3H s, H-22), 2.57 (6H s, $N(CH_3)_2$), 3.45 (3H s, 2‴-OCH_3), 3.59 (3H s, 3‴-OCH_3), 4.16 (1H d, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.54 (1H d, $J_{1''',2'''}$ = 8.0 Hz, H-1‴), 5.0 (1H b, H-15), 5.06 (1H b, H-1″), 5.83 (1H d, $J_{13,14}$ = 9.5 Hz, H-13), 6.23 (1H d, $J_{10,11}$ = 13.5 Hz, H-10), 7.1 ~ 7.6 (H-11 and aromatic protons).

10,11-Dihydro-11-phenylthiotylosin Diphenyl Dithioacetal (**6**): [Method A]

Compound **5** (1.0 g) and thiophenol (1.0 ml) were dissolved in triethylamine (20 ml), and the solution was refluxed for 4 hours under nitrogen atmosphere. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$ - MeOH - conc NH_4OH, 40: 1: 0.05) to yield compound **6** (440 mg, yield 40.3%). TLC Rf 0.62 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −58.3° (*c* 1.0, MeOH); EI-mass *m/z* 718, 702 (aglycone), 191 (mycinose), 174 (mycaminose); ^1H NMR δ 1.63 (3H s, H-22), 2.46 (6H s, $N(CH_3)_2$), 3.43 (3H s, 2‴-OCH_3), 3.56 (3H s, 3‴-OCH_3), 4.34 (1H d, $J_{1''',2'''}$ = 7.5 Hz, H-1‴), 4.67 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 4.8 (1H b, H-15), 5.00 (1H b, H-1″), 7.2 (m, aromatic protons).

11-*p*-Aminophenylthio-10,11-dihydrotylosin Diphenyl Dithioacetal (**7**)

Compound **7** was obtained from **5** and *p*-aminothiophenol according to method A. Yield 58.5%. TLC Rf 0.51 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −57.6° (*c* 0.1, MeOH); EI-mass *m/z* 717 (aglycone), 191 (mycinose), 174 (mycaminose); ^1H NMR δ 1.66 (3H s, H-22), 4.60 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 6.42 and 6.95 (each 2H d, J = 8.0 Hz, $-SC_6H_4NH_2$), 7.3 (m, aromatic protons of diphenyl dithioacetal).

10,11-Dihydro-11-ethylthiotylosin Diphenyl Dithioacetal (**8**)

Compound **8** was obtained from **5** and ethanethiol according to method A. Yield 71.1%. TLC Rf 0.61 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −53.1° (*c* 1.0, MeOH); EI-mass *m/z* 845 (aglycone+mycinose), 6.54 (aglycone), 191 (mycinose), 145 (mycarose); ^1H NMR δ 1.63 (3H s, H-22), 7.3 (m, aromatic protons).

10,11-Dihydro-11-*p*-methylphenylthiotylosin Diphenyl Dithioacetal (**9**)

Compound **9** was obtained from **5** and *p*-methylthiophenol according to method A. Yield 38.7%. TLC Rf 0.59 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −54.9° (*c* 1.0, MeOH); EI-mass *m/z* 1,241 (M^+), 1,096 (M^+ − mycarose), 191, 175 (mycinose), 174 (mycaminose), 161, 145 (mycarose); ^1H NMR δ 1.63 (3H s, H-22), 2.25 (3H s, $-SC_6H_4CH_3$), 4.63 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 6.9 ~ 7.5 (m, aromatic protons).

11-*p*-Chlorophenylthio-10,11-dihydrotylosin Diphenyl Dithioacetal (**10**)

Compound **10** was obtained from **5** and *p*-chlorothiophenol according to method A. Yield 37.2%. TLC Rf 0.55 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −65.4° (*c* 1.0, MeOH); EI-mass *m/z* 927 (aglycone+mycinose), 191, 175 (mycinose), 174 (mycaminose), 161 (mycarose); ^1H

NMR δ 1.63 (3H s, H-22), 4.69 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 7.0~7.4 (m, aromatic protons).

10,11-Dihydro-11-*p*-methoxyphenylthiotylosin Diphenyl Dithioacetal (**11**)

Compound **11** was obtained from **5** and *p*-methylthiophenol according to method A. Yield 37.3%. TLC Rf 0.57 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −52.9° (*c* 1.0, MeOH); EI-mass *m/z* 764 (aglycone), 191, 175 (mycinose), 174 (mycaminose), 161, 145 (mycarose); ^1H NMR δ 1.64 (3H s, H-22), 3.68 (3H s, $-SC_6H_4OCH_3$), 4.56 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 6.5~7.4 (m, aromatic protons).

10,11-Dihydro-11-phenylthiodemycarosyltylosin Dimethyl Acetal (**12**)

Compound **12** was obtained from **4** and thiophenol according to method A. Yield 34.9%. TLC Rf 0.68 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −8.4° (*c* 1.0, MeOH); EI-mass *m/z* 927 (M^+), 753 (M^+ −mycaminose), 190, 174 (mycaminose); ^1H NMR δ 1.63 (3H s, H-22), 2.49 (6H s, $N(CH_3)_2$), 3.23 (6H s, 20-$(OCH_3)_2$), 3.44 (3H s, 2″-OCH_3), 3.56 (3H s, 3″-OCH_3), 4.31 (1H d, $J_{1',2'}$ = 7.0 Hz, H-1′), 4.40 (1H d, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.9 (2H b, H-13 and H-15), 7.3 (m, aromatic protons).

11-*p*-Aminophenylthio-10,11-dihydrodemycarosyltylosin Dimethyl Acetal (**13**)

Compound **13** was obtained from **4** and *p*-aminothiophenol according to method A. Yield 33.6%. TLC Rf 0.64 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −16.0° (*c* 0.1, MeOH); EI-mass *m/z* 768 (M^+ −mycaminose), 190 (mycaminose), 175 (mycinose); ^1H NMR δ 1.69 (3H s, H-22), 4.77 (1H bd, $J_{13,14}$ = 11.0 Hz, H-13), 6.56, 7.12 (each 2H d, *J* = 8.0 Hz, aromatic protons).

10,11-Dihydro-11-ethylthiodemycarosyltylosin Dimethyl Acetal (**14**)

Compound **14** was obtained from **4** and ethanethiol according to method A. Yield: 79.8%. TLC Rf 0.68 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −13.4° (*c* 0.5, MeOH); EI-mass *m/z* 879 (M^+), 688 (M^+ −mycinose), 174 (mycaminose); ^1H NMR δ 1.67 (3H s, H-22), 5.20 (1H bd, $J_{13,14}$ = 9.5 Hz, H-13).

10,11-Dihydro-11-(2-hydroxyethylthio)demycarosyltylosin Dimethyl Acetal (**15**)

Compound **15** was obtained from **4** and 2-mercaptoethanol according to method A. Yield 34.5%. TLC Rf 0.58 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −12.0° (*c* 0.1, MeOH); EI-mass *m/z* 530 (aglycone), 174 (mycaminose); ^1H NMR δ 1.83 (3H s, H-22), 5.10 (1H bd, $J_{13,14}$ = 8.5 Hz, H-13).

10,11-Dihydro-11-phenylthiotylosin (**16**) [Method B]

Compound **6** (400 mg), mercury (II) oxide (red) (310 mg) and boron trifluoride etherate (0.2 ml) were dissolved in 15% aq THF (4 ml), and the mixture was stirred for 1 hour at room temperature under nitrogen atmosphere. The reaction mixture was poured into saturated sodium carbonate solution, and extracted with ether. The organic layer was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was subjected to silica gel column chromatography ($CHCl_3$ - MeOH - conc NH_4OH, 35: 1: 0.05) to give **16** (185 mg, yield 55.4%). TLC Rf 0.49 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −40.8° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 262 (5,000); EI-mass *m/z* 864 (M^+ −mycarose), 191 (mycinose), 174 (mycaminose); ^1H NMR δ 1.63 (3H s, H-22), 2.47 (6H s, $N(CH_3)_2$), 3.41 (3H s, 2‴-OCH_3), 3.54 (3H s, 3‴-OCH_3), 4.18 (1H d, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.36 (1H d, $J_{1''',2'''}$ = 7.5 Hz, H-1‴), 4.84 (1H d, $J_{13,14}$ = 9.5 Hz, H-13), 4.90 (1H b, H-15), 4.99 (1H bd, $J_{1'',2''}$ = 3.0 Hz, H-1″), 7.2 (m, aromatic protons), 9.75 (1H s, H-20), ^{13}C NMR Table 1.

11-*p*-Aminophenylthio-10,11-dihydrotylosin (**17**)

Compound **17** was obtained from **7** according to method B. Yield 17.9%. TLC Rf 0.41 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −59.6° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 274 (16,000); EI-mass *m/z* 531 (aglycone), 191 (mycinose), 174 (mycaminose); ^1H NMR δ 1.75 (3H s, H-22), 6.55, 7.13 (each 2H d, aromatic protons), 9.70 (1H s, H-20).

10,11-Dihydro-11-ethylthiotylosin (**18**)

Compound **18** was obtained from **8** according to method B. Yield 29.8%. TLC Rf 0.49 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −50.0° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 285 (7,100); EI-mass *m/z* 641 (aglycone+mycaminose), 484, 452 (aglycone), 175 (mycinose), 174

(mycaminose); ^{1}H NMR δ 1.64 (3H s, H-22), 9.66 (1H s, H-20); ^{13}C NMR Table 1.

10,11-Dihydro-11-*p*-methylphenylthiotylosin (**19**)

Compound **19** was obtained from **9** according to method B. Yield 28.1%. TLC Rf 0.51 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −46.0° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 263 (6,700); EI-mass *m/z* 894 (M^+ −mycarose), 706 (aglycone+mycinose), 318 (mycarosylmycaminose), 174 (mycaminose), 145 (mycarose); ^{1}H NMR δ 1.66 (3H s, H-22), 2.30 (3H s, $-SC_6H_4CH_3$), 4.9 (2H b, H-13 and H-15), 7.03, 7.23 (each 2H d, J = 8.0 Hz, aromatic protons), 9.65 (1H s, H-20); ^{13}C NMR Table 1.

11-*p*-Chlorophenylthio-10,11-dihydrotylosin (**20**)

Compound **20** was obtained from **10** according to method B. Yield 43.7%. TLC Rf 0.51 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −53.6° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 266 (8,500); EI-mass *m/z* 898 (M^+ −mycarose), 725 (aglycone+mycinose), 566 (aglycone), 191, 175 (mycinose), 174 (mycaminose); ^{1}H NMR δ 1.66 (3H s, H-22), 4.62 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 7.26 (s, aromatic protons), 9.67 (1H s, H-20); ^{13}C NMR Table 1.

10,11-Dihydro-11-*p*-methoxyphenylthiotylosin (**21**)

Compound **21** was obtained from **11** according to method B. Yield 50.6%. TLC Rf 0.50 ($CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.05); $[\alpha]_D^{29}$ −49.7° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 262 (5,400), 231 (10,200); EI-mass *m/z* 562, 530 (aglycone), 318 (mycarosylmycaminose), 174 (mycaminose); ^{1}H NMR δ 1.69 (3H s, H-22), 3.76 (3H s, $-SC_6H_4OCH_3$), 4.78 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 6.78, 7.30 (each 2H d, J = 9.0 Hz, aromatic protons), 9.66 (1H s, H-20); ^{13}C NMR Table 1.

10,11-Dihydro-11-phenylthiodemycarosyltylosin (**22**) [Method C]

Compound **12** (40 mg) was dissolved in a mixture of 0.1 N HCl and CH_3CN (2.5: 1) (1 ml), and the solution was allowed to stand for 1 hour at room temperature. The reaction mixture was poured into sodium bicarbonate solution, and was extracted with $CHCl_3$. The organic layer was dried over anhydrous sodium sulfate, and dried up *in vacuo*. The residue was purified by preparative silica gel TLC ($CHCl_3$ - MeOH - conc NH_4OH, 8: 1: 0.05) to yield **22** (25 mg, yield 65.8%). TLC Rf 0.64 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −11.4° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 264 (4,700); EI-mass *m/z* 881 (M^+), 190, 174 (mycinose); ^{1}H NMR δ 1.66 (3H s, H-22), 2.47 (6H s, $N(CH_3)_2$), 3.45 (3H s, 2″-OCH_3), 3.54 (3H s, 3″-OCH_3), 4.25 (1H d, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.37 (1H d, $J_{1'',2''}$ = 7.5 Hz, H-1″), 4.91 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 4.8~5.0 (1H b, H-15), 7.2~7.3 (m, aromatic protons), 9.66 (1H s, H-20); ^{13}C NMR Table 1.

11-*p*-Aminophenylthio-10,11-dihydrodemycarosyltylosin (**23**)

Compound **23** was obtained from **13** according to method C. Yield 84.1%. TLC Rf 0.61 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −18.3° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 271 (13,000); EI-mass *m/z* 706 (M^+ −mycaminose), 531 (aglycone), 191, 175 (mycinose), 190, 174 (mycaminose); ^{1}H NMR δ 1.69 (3H s, H-22), 4.77 (1H bd, $J_{13,14}$ = 10.5 Hz, H-13), 6.56, 7.16 (each 2H d, J = 8.0 Hz, aromatic protons), 9.67 (1H s, H-20).

10,11-Dihydro-11-ethylthiodemycarosyltylosin (**24**)

Compound **24** was obtained from **14** according to method C. Yield 92.3%. TLC Rf 0.67 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −14.9° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 282 (2,700); EI-mass *m/z* 833 (M^+), 642 (M^+ −mycinose), 174 (mycaminose); ^{1}H NMR δ 1.67 (3H s, H-22), 5.17 (1H bd, $J_{13,14}$ = 9.5 Hz, H-13), 9.66 (1H s, H-20); ^{13}C NMR Table 1.

10,11-Dihydro-11-(2-hydroxyethylthio)demycarosyltylosin (**25**)

Compound **25** was obtained from **15** according to method C. Yield 80.8%. TLC Rf 0.58 ($CHCl_3$ - MeOH - conc NH_4OH, 6: 1: 0.05); $[\alpha]_D^{29}$ −34.4° (*c* 0.5, MeOH); UV λ_{max}^{MeOH} nm (ε) 285 (2,100); EI-mass *m/z* 468 (aglycone), 174 (mycaminose); ^{1}H NMR δ 1.81 (3H s, H-22), 5.08 (1H bd, $J_{13,14}$ = 9.0 Hz, H-13), 5.1 (1H b, H-15), 9.67 (1H s, H-20); ^{13}C NMR Table 1.

Acknowledgment

The authors thank Mr. R. MASUMA for determination of antimicrobial activities, and Miss M. KAWASHIMA for helpful technical assistance.

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