19-DEFORMYL-4′-DEOXYDESMYCOSIN (TMC-016): SYNTHESIS AND BIOLOGICAL PROPERTIES OF A UNIQUE 16-MEMBERED MACROLIDE ANTIBIOTIC

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19-Deformyl-4′-deoxydesmycosin was synthesized by the following synthetic route: 19-Deformylation of desmycosin, 3,2′,4″-tri-*O*-trimethylsilylation, 4′-*O*-sulfonylation, 4′-iodination, reductive deiodination and 3,2′,4″-tri-*O*-detrimethylsilylation.

Deformylation of the aldehyde group at the C-19 position was achieved by two different methods: A) A simple one-step deformylation using Wilkinson's catalyst ($(Ph_3P)_3RhCl$). B) Reductive decarboxylation of the 19-carboxyl derivative following $NaClO_2$ oxidation of the aldehyde.

19-Deformyl-4′-deoxydesmycosin showed very strong antimicrobial activity *in vitro* and *in vivo*.

Desmycosin (demycarosyltylosin), a 16-membered macrolide antibiotic possessing an aldehyde function is obtained by the mild acidic hydrolysis of tylosin[1] and has good antimicrobial activity *in vitro* against Gram-positive bacteria and *Mycoplasma* but almost no activity *in vivo* when orally administered.

Mycinamicins, a group of macrolides lacking the aldehyde group, have been isolated from fermentations by other workers in our laboratory[2] and have shown higher antibacterial activity than other clinically used macrolide antibiotics. Recently, a large number of derivatives of desmycosin and tylosin have been reported and some biological improvements have been made, especially by modification of the aldehyde function[3~5]. We have reported deformylation of the aldehyde moiety of desmycosin by utilizing Wilkinson's catalyst ($(Ph_3P)_3RhCl$)[3] and noted an improvement in its biological activities.

We now report the synthesis and biological properties of a novel macrolide antibiotic, 19-deformyl-4′-deoxydesmycosin.

Experimental

General Methods

MP's were not corrected. Optical rotations were determined with a Horiba SEPA-200 polarimeter. UV and IR spectra were recorded on Shimadzu UV-365 and Hitachi 260-50 IR spectrometers, respectively. NMR spectra were measured in $CDCl_3$ using Jeol FX-90 and GSX-400 spectrometers, with TMS as an internal standard. Mass spectra were obtained with a Jeol JMS-D300 mass spectrometer. Column chromatography was performed on Silica gel 60 (Merck, Art. No. 7734 or 9385 for flash chromatography). Rf values were measured on Silica gel 60 (Merck, Art. No. 5715) plates using the following TLC systems: A) Benzene - acetone (3 : 1), B) chloroform - methanol (5 : 1), C) chloroform - methanol - 28% concd ammonia (15 : 1 : 0.1). Spots on TLC plate were detected by UV absorption and by heating at 100°C after spraying with concd sulfuric acid.

Synthesis of 19-Deformyl-4′-deoxydesmycosin (**10**)

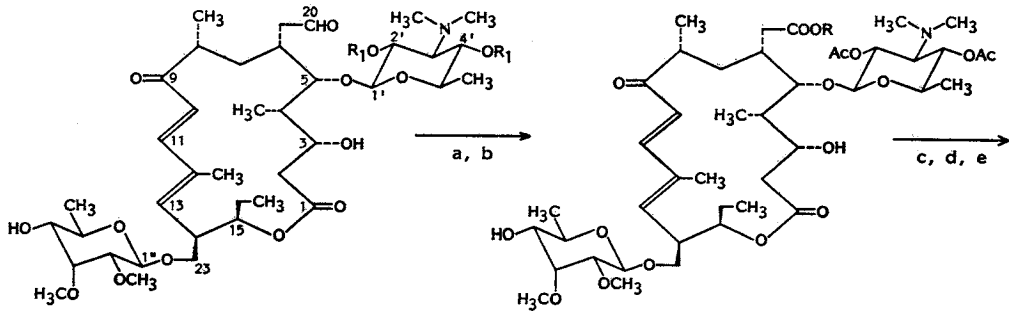
The synthesis of compound **10** is shown in Fig. 1. Desmycosin (**1**) (10 g) and $(Ph_3P)_3RhCl$ (12 g) were dissolved in 150 ml of dry benzene and refluxed for 6 hours under an argon atmosphere. The reaction mixture was evaporated and washed with acetone, then the resulting yellow precipitate was filtered off and the filtrate was evaporated under reduced pressure. The residue was dissolved in benzene (100 ml) and extracted three times with 0.4 N aqueous hydrochloric acid (150 ml each). The extract was washed with *n*-hexane, then adjusted to pH 9 to 9.5 by adding concd ammonia and extracted with chloroform (500 ml). The extract was filtered through Whatman 1PS filter paper and then evaporated *in vacuo* to obtain crude 19-deformyldesmycosin (**6**) (7.5 g). To thus obtained compound **6** (5 g) dissolved in dry dichloromethane (100 ml), dry pyridine (4.75 ml) and chlorotrimethylsilane (6.8 ml) were added under ice-cooling and the mixture was stirred for 2 hours at 5 to 10°C. Ice-water (200 ml) was added and the reaction mixture was adjusted to pH 9 to 9.5 with 7% aqueous ammonia, and extracted with chloroform (200 ml). The extract was washed with brine, dried over anhydrous magnesium sulfate and then evaporated *in vacuo* to yield a mixture of 19-deformyl-3,2′,4″-tri-*O*-trimethylsilyldesmycosin (**7**) and a small amount of 19-deformyl-3,2′,4′,4″-tetra-*O*-trimethylsilyldesmycosin. To the mixture (5.5 g) dissolved in dry pyridine (55 ml), methane sulfonylchloride (2.1 ml) was added under ice-cooling and the mixture was stirred at 0 to 5°C for 4 hours. The reaction mixture was poured into ice-water (900 ml) and adjusted to pH 9 to 9.5 with 7% aqueous ammonia. The resulting precipitate was collected by filtration and dissolved in chloroform (100 ml) then washed with brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo* to obtain crude 19-deformyl-4′-*O*-methanesulfonyl-3,2′,4″-tri-*O*-trimethylsilyldesmycosin (**8**) as the major product. To the above crude mixture dissolved in dry methyl ethyl ketone (50 ml), sodium iodide (2.2 g) was added, and the mixture was stirred at 80°C for 1.5 hours. The reaction mixture was concentrated under reduced pressure and added to water (200 ml), adjusted to pH 9 to 9.5 by adding 7% aqueous ammonia, then extracted with chloroform (200 ml). The organic layer was washed with aqueous sodium thiosulfate and brine, filtered through Whatman 1PS filter paper and evaporated *in vacuo*. The mixture was dissolved in 0.2 N aqueous hydrochloric acid (100 ml) - acetonitrile (100 ml) and stirred for 2 hours at room temperature. The reaction mixture was adjusted to pH 9 to 9.5 with concd ammonia and extracted twice with chloroform (200 ml each). The extract was washed with brine and dried over anhydrous magnesium sulfate, then evaporated to dryness under reduced pressure to give a crude mixture of 19-deformyl-4′-deoxy-4′-iododesmycosin (**9**) and a small amount of 19-deformyldesmycosin (**6**). The residue was dissolved in a small amount of chloroform and applied to a silica gel column (silica gel; 80 g). Elution was carried out with a mixture of chloroform and methanol (100 : 1, 800 ml then 10 : 1). Each fraction (100 ml) was examined by silica gel TLC and the fractions showing a spot at Rf 0.49 (system C) were collected and evaporated to give compound **9** (3.5 g). The white powder (760 mg) obtained from the fractions showing Rf 0.34 (system C) was recovered as 19-deformyldesmycosin (**6**). Compound **9** thus obtained was dissolved in dry benzene (50 ml) and reacted with tri(*n*-butyl)stannane (2.1 ml) in the presence of 2,2′-azobis(isobutyronitrile) (15 mg) under an argon atmosphere at 80°C for 1 hour. The reaction mixture was extracted three times with 0.4 N aqueous hydrochloric acid (100 ml each). The combined aqueous layer was washed with *n*-hexane, then adjusted to pH 9 to 9.5 with concd ammonia and extracted with chloroform (300 ml). The extract was filtered through Whatman 1PS filter paper then evaporated *in vacuo* and purified by flash column chromatography (silica gel; 60 g), developing with chloroform - methanol (60 : 1) to yield a white crystalline powder of 19-deformyl-4′-deoxydesmycosin (**10**) (2.72 g, yield 55.6% from **6**): MP 118~120°C; Rf 0.49 (system C); $[\alpha]_D^{22}$ +9.5° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε) 281 (20,000); IR (KBr) cm^{-1} 3480, 2970, 2930, 1710, 1675, 1620, 1590, 1455, 1375, 1355, 1310, 1270, 1125, 1070, 980, 955, 890, 855, 835, 810, 750; chemical ionization (CI)-MS *m/z* 728 (M+H), 158; ^1H and ^{13}C NMR data are shown in Tables 1 and 2 respectively.

Anal Calcd for $C_{38}H_{65}NO_{12}$(727.93): C 62.70, H 9.00, N 1.92.
Found: C 62.40, H 9.07, N 1.86.

Preparation of 19-Deformyldesmycosin (**6**)

To desmycosin (**1**) (2.5 g) dissolved in dry dichloromethane (25 ml), acetic anhydride (1.53 ml)

Fig. 1. Synthesis of 19-deformyl-4′-deoxydesmycosin (**10**).

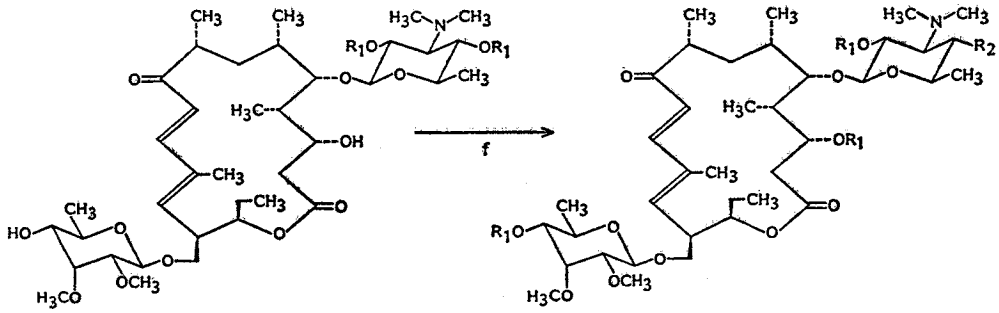


Desmycosin (**1**) R_1=H

2 R_1=Ac

3 R=H

4 R= 



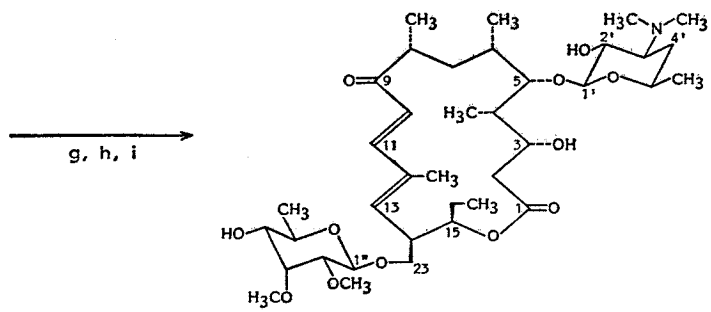
5 R_1=Ac

6 R_1=H

7 R_1=TMS R_2=OH

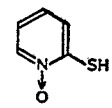
8 R_1=TMS R_2=OMS

9 R_1=TMS R_2=H



19-Deformyl-4′-deoxydesmycosin (**10**)

a) Ac_2O, CH_2Cl_2, b) $NaClO_2$ - H_2NSO_3H, aq acetone,

c) $ClCOOCH_2CH(CH_3)_2$ - triethylamine - dimethoxyethane  , 0～−3°C,

d) thiophenol, 85°C, e) MeOH, 55°C, f) Me_3SiCl - pyridine, 0～5°C, g) $MeSO_3Cl$ - pyridine; NaI, MeCOEt, 80°C, h) aq CH_3CN - HCl, i) Bu_3SnH, benzene, 80°C.

was added and the mixture was stirred for 1 hour at room temperature. The reaction mixture was added to ice-water (50 ml), adjusted to pH 9 to 9.5 by adding 7% aqueous ammonia, and then extracted with chloroform (50 ml). The extract was filtered through Whatman 1PS filter paper and evaporated to dryness to obtain 2',4'-di-*O*-acetyldesmycosin (**2**) (2.6 g): CI-MS *m/z* 856 (M+H), 258; ^{1}H NMR δ 1.79 (s, 12-CH_3), 2.04 (s, CH_3CO), 2.05 (s, CH_3CO), 2.34 (s, 3'-$N(CH_3)_2$), 3.49 (s, 2''-OCH_3), 3.61 (s, 3''-OCH_3), 4.31 (d, 1'-H), 4.56 (d, 1''-H), 4.73 (dd, 4'-H), 4.88 (dd, 2'-H), 5.00 (dt, 15-H), 5.91 (d, 13-H), 6.29 (d, 10-H), 7.33 (d, 11-H), 9.67 (s, CHO).

To compound **2** dissolved in acetone (15 ml) 0.3 N aqueous sulfamic acid (20.3 ml) and 0.2 N aqueous sodium chlorite (18 ml) were added under ice-cooling and the mixture was stirred for 1 hour at room temperature. The reaction mixture was neutralized by adding 7% aqueous ammonia and extracted three times with chloroform (50 ml each). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo* to give crude 2',4'-di-*O*-acetyl-19-carboxy-19-deformyldesmycosin (**3**). The residue was chromatographed on a silica gel column (50 g) and developed with chloroform - methanol (7 : 1) to yield a white powder of compound **3** (2.12 g, yield 75.1%): MP 134~137°C; Rf 0.66 (system B); $[\alpha]_D^{20}$ −20.1° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε) 282 (21,600); fast atom bombardment (FAB)-MS *m/z* 872 (M+H); IR (KBr) cm^{-1} 3460, 2960, 2925, 1720, 1680, 1590, 1450, 1375, 1310, 1230, 1160, 1050, 980, 910, 840, 810; ^{1}H NMR δ 1.78 (s, 12-CH_3), 2.04 (s, CH_3CO), 2.06 (s, CH_3CO), 2.34 (s, 3'-$N(CH_3)_2$), 3.46 (s, 2''-OCH_3), 3.61 (s, 3''-OCH_3), 4.33 (d, 1'-H), 4.56 (d, 1''-H), 5.90 (d, 13-H), 6.27 (d, 10-H), 7.35 (d, 11-H), no CHO signal.

Anal Calcd for $C_{43}H_{69}NO_{17}$: C 59.23, H 7.98, N 1.61.
Found: C 59.00, H 8.01, N 1.59.

To compound **3** (500 mg) dissolved in dimethoxyethane (5 ml), triethylamine (0.08 ml) and isobutyl chloroformate (0.0745 ml) were added under ice-salt-cooling and kept at 0 to −3°C for 5 minutes and then *N*-hydroxypyridine-2-thione (87.5 mg) was added and the mixture was stirred for 5 minutes then allowed to react at room temperature for 15 minutes. To this reaction mixture thiophenol (0.118 ml) and 2,2'-azobis(isobutyronitrile) (19 mg) were added and the mixture was heated at 90°C under an argon atmosphere for 2 hours. The reaction mixture was concentrated *in vacuo* and dissolved in chloroform (100 ml) then washed with saturated aqueous sodium bicarbonate. The organic layer was separated and dehydrated by a Whatman 1PS phase-separator and evaporated to dryness under reduced pressure to obtain crude 2',4'-di-*O*-acetyl-19-deformyldesmycosin (**5**). The residue was purified by silica gel flash column chromatography (silica gel; 20 g), developing with a mixture of benzene and acetone (12 : 1) to yield a white powder of compound **5** (255 mg, yield 53.7% from **3**). This compound **5** dissolved in methanol (5 ml) was stirred at 55°C for 16 hours and evaporated *in vacuo* to afford 19-deformyldesmycosin (**6**) in quantitative yield: MP 118~121°C; Rf 0.34 (system C); $[\alpha]_D^{20}$ +2.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε) 282 (22,500); CI-MS *m/z* 744 (M+H), 174; IR (KBr) cm^{-1} 3450, 2970, 2930, 1710, 1670, 1620, 1590, 1460, 1410, 1375, 1355, 1310, 1260, 1165, 1070, 1005, 980, 955, 900, 860, 835, 810, 700; ^{1}H NMR δ 1.79 (s, 12-CH_3), 2.51 (s, 3'-$N(CH_3)_2$), 3.48 (s, 2''-OCH_3), 3.62 (s, 3''-OCH_3), 4.29 (d, 1'-H), 4.56 (d, 1''-H), 4.98 (m, 15-H), 5.83 (d, 13-H), 6.25 (d, 10-H), 7.26 (d, 11-H).

Anal Calcd for $C_{38}H_{65}NO_{13}$(743.93): C 61.35, H 8.81, N 1.88.
Found: C 61.07, H 8.51, N 1.60.

Isolation of 19-Deformyl-3,2',4''-tri-*O*-trimethylsilyldesmycosin (**7**)

A crude mixture (550 mg) of compound **7** as obtained during the synthesis of compound **10** described above, was applied to a silica gel (6 g) column and eluted with benzene - acetone (30 : 1). The fractions (10 ml) showing a spot at Rf 0.43 (system A) were collected and evaporated *in vacuo* to obtain amorphous compound **7** (230 mg): $[\alpha]_D^{20}$ −3.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε) 283 (21,600); CI-MS *m/z* 960 (M+H), 246; IR (KBr) cm^{-1} 3450, 3050, 2960, 2940 (sh), 1740, 1680, 1590, 1450, 1380, 1360 (sh), 1320, 1260, 1170, 1100, 1080 (sh), 1040 (sh), 980 (sh), 960, 900 (sh), 865, 840, 750; ^{1}H NMR δ 0.03 (s, 3-O-trimethylsilyl (TMS)), 0.14 (s, 4''-O-TMS), 0.19 (s, 2'-O-TMS), 1.76 (s, 12-CH_3), 2.48 (s, 3'-$N(CH_3)_2$), 3.45 (s, 2''-OCH_3), 3.57 (s, 3''-OCH_3), 4.18 (d, 1'-H), 4.57 (d, 1''-H), 4.86 (m, 15-H), 5.85 (d, 13-H), 6.22 (d, 10-H), 7.30 (d, 11-H).

Anal Calcd for $C_{47}H_{89}NO_{13}Si_3$: C 58.77, H 9.34, N 1.46.
Found: C 58.47, H 9.15, N 1.17.

Results and Discussion

Synthesis of 19-Deformyl-4′-deoxydesmycosin (10)

Desmycosin (**1**) was refluxed in benzene with a stoichiometric amount of Wilkinson's catalyst ($(Ph_3P)_3RhCl$) to obtain 19-deformyldesmycosin (**6**) in 70 to 80% yield as a single isolatable product. This very simple and high-yield deformylation process is the most desirable method, but Wilkinson's catalyst is too expensive to use for large scale preparation. Therefore a new route for the deformylation was investigated. Reductive decarboxylation[6] by the Barton reaction of the 19-carboxyl derivative prepared from compound **1** was accomplished in the following manner.

The hydroxyl groups at the C-2′ and C-4′ positions of compound **1** were easily acetylated with acetic anhydride at room temperature to afford a quantitative yield of 2′,4′-di-*O*-acetyldesmycosin (**2**). The aldehyde at the C-19 position in compound **2** was readily oxidized to the corresponding carboxyl group at ambient temperature using $NaClO_2$ as an oxidant and sulfamic acid as a scavenger[7], to give 2′,4′-di-*O*-acetyl-19-deformyl-19-carboxydesmycosin (**3**) in 80 to 90% yield. Compound **3** was converted *in situ* to the decarboxylated derivative, 2′,4′-di-*O*-acetyl-19-deformyldesmycosin (**5**), in good yield by heating the thioester derivative (**4**) prepared from compound **3** and *N*-hydroxypyridine-2-thione with thiophenol or triphenylmethylmercaptan as a hydrogen donor. Deacetylation of compound **5** was achieved in hot methanol to give compound **6** in quantitative yield. Successive in-

Table 1. ^1H NMR chemical shifts[a] for 19-deformyl-4′-deoxydesmycosin (**10**) and desmycosin (**1**).

	Chemical shift (ppm)			Chemical shift (ppm)	
	10	**1**		**10**	**1**
2-H_a	2.49	2.49	1′-H	4.24	4.26
2-H_b	1.97	1.94	2′-H	3.23	3.49
3-H	3.78	3.84	3′-H	2.4	2.36
4-H	1.66	1.6	4′-H_a	1.64	3.06
5-H	3.55	3.73	4′-H_b	1.25	—
6-H	1.60	2.16	5′-H	3.46	3.27
7-H_a	1.65	1.6	5′-CH_3(6′)	1.22	1.27
7-H_b	1.52	1.5	3′-$N(CH_3)_2$(7′,8′)	2.27	2.50
8-H	2.67	2.61	1″-H	4.56	4.56
10-H	6.30	6.26	2″-H	3.02	3.02
11-H	7.29	7.32	3″-H	3.75	3.75
13-H	5.86	5.91	4″-H	3.18	3.18
14-H	2.96	2.96	5″-H	3.50	3.50
15-H	4.95	4.99	5″-CH_3(6″)	1.26	1.27
16-H_a	1.86	1.88	2″-OCH_3(7″)	3.48	3.49
16-H_b	1.62	1.6	3″-OCH_3(8″)	3.62	3.62
16-CH_3(17)	0.93	0.94			
4-CH_3(18)	1.10	1.01			
6-CH_3(19)	1.08	—			
19-H_a	—	2.92			
19-H_b	—	2.47			
20-H	—	9.70			
8-CH_3(21)	1.19	1.21			
12-CH_3(22)	1.77	1.79			
23-H_a	4.00	4.00			
23-H_b	3.55	3.56			

[a] Values in ppm from Me_4Si; determined from a 2D homonuclear shift-correlated (COSY) experiment.

Table 2. ^{13}C NMR assignment for 19-deformyl-4′-deoxydesmycosin (**10**) and desmycosin (**1**).

Carbon	Chemical shift (ppm)		Carbon	Chemical shift (ppm)	
	10	**1**		**10**	**1**
1	174.5	173.8	1′	104.7	104.1
2	39.5	39.4	2′	70.5	70.9
3	67.9	68.1	3′	65.8	70.6
4	40.7	40.3	4′	28.4	70.2
5	85.8	81.3	5′	69.3	73.3
6	31.6	32.1	5′-CH_3(6′)	21.1	17.8
7	34.8	32.8	3′-$N(CH_3)_2$(7′,8′)	40.3	41.7
8	45.0	45.1	1″	101.1	101.0
9	204.3	203.1	2″	81.9	81.9
10	119.2	118.7	3″	79.8	79.8
11	147.2	148.0	4″	72.7	72.7
12	135.1	134.8	5″	70.6	70.6
13	141.4	142.2	5″-CH_3(6″)	17.8	17.8
14	44.9	44.7	2″-OCH_3(7″)	59.7	59.7
15	75.1	75.1	3″-OCH_3(8″)	61.7	61.7
16	25.4	25.5			
16-CH_3(17)	9.6	9.7			
4-CH_3(18)	9.0	9.0			
6-CH_3(19)	17.6	—			
19	—	43.8			
20	—	202.8			
8-CH_3(21)	17.6	17.4			
12-CH_3(22)	13.0	13.0			
23	69.1	69.1			

Assignments are based on a ^{13}C-^1H shift-correlated 2D NMR spectrum.

vestigation showed that selective trimethylsilylation of the three hydroxyl groups at the C-3, C-2′ and C-4″ positions of compound **6** was performed by reacting with 6 to 8 equivalents of chlorotrimethylsilane in the presence of pyridine in an excellent yield. Since the 19-deformyl-3,2′,4″-tri-*O*-trimethylsilyldesmycosin (**7**) thus obtained was not very stable, the reaction mixture was carried forward to the next step without further purification.

Deoxygenation of the C-4 hydroxyl group in mycaminose has already been accomplished[8,9]. In our hands, compound **7** was reacted with methanesulfonyl chloride in the presence of triethylamine or pyridine at 0 to 5°C to give the corresponding 4′-*O*-sulfonyl derivative (**8**). Successive iodination of compound **8** with sodium iodide and reductive deiodination with tri(*n*-butyl)stannane, gave the 4′-deoxy derivative (**9**). Treatment of compound **9** with aqueous acid resulted in removal of the trimethylsilyl group and yielded compound **10** (yield 41.6% from **1**).

The structure of compound **10** was elucidated by mass spectrometry, ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectral evidence including ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C COSY spectra.

In Vitro Activity

The antimicrobial activity (MIC) of compound **10** was compared with that of 19-deformyldesmycosin (**6**) and erythromycin and clindamycin. As shown in Table 3, among the macrolides tested, compound **10** showed the best activity against *Staphylococcus aureus* including a macrolide-resistant strain, but showed less activity against *Enterococcus faecalis* and *Streptococcus agalactiae*. Com-

Table 3. Antimicrobial activity of compounds **10** and **6** compared with that of desmycosin (**1**) and erythromycin (EM).

Test organism	MIC (μg/ml) (10^6 cells/ml)			
	10	**6**	**1**	EM
Staphylococcus aureus ATCC 6538P	0.10	0.20	0.39	0.20
S. aureus MS353	0.10	0.20	0.39	0.20
S. aureus MS353 C36*	0.10	0.20	0.39	>100
S. aureus MS353 AO***	>100	>100	>100	>100
S. aureus 0175***	0.39	3.13	1.56	>100
S. aureus 0126**	0.39	0.78	1.56	>100
S. aureus Smith	0.10	0.20	0.78	0.20
S. epidermidis sp-al-1	≤0.05	≤0.05	0.20	0.10
Streptococcus pyogenes N.Y. 5	≤0.05	≤0.05	0.10	≤0.05
S. pyogenes 1022***	>100	>100	>100	>100
S. pyogenes S-23	≤0.05	≤0.05	≤0.05	≤0.05
S. agalactiae 1020	3.13	3.13	0.39	0.10
S. pneumoniae 10[a]	0.39	0.78	0.20	≤0.05
S. pneumoniae DP-III[a]	1.56	1.56	NT	≤0.05
Enterococcus faecalis 1501	6.25	12.5	0.78	0.39
Micrococcus luteus ATCC 9341	≤0.05	≤0.05	0.10	≤0.05
Corynebacterium diphtheriae P.W. 8	0.10	0.20	≤0.05	≤0.05
Bacillus subtilis ATCC 6633	0.20	0.39	0.39	0.10
Haemophilus influenzae 1322[b]	1.56	3.13	NT	1.56
Escherichia coli NIHJ JC-2	>100	>100	>100	>100
Klebsiella pneumoniae NCTC 9632	>100	>100	>100	50
Mycoplasma pneumoniae Mac[c]	0.0063	0.0125	NT	0.025
M. pneumoniae FH[c]	0.0032	0.0125	NT	0.0125

Media: STA (Nissui). [a] STA supplemented with 5% horse blood. [b] BHIA supplemented with 5% Bacto-Fildes enrichment (Difco). [c] PPLO broth supplemented with 20% horse serum.
* EM-resistant. ** EM-oleandomycin-resistant. *** Macrolide-resistant.
NT: Not tested.

Table 4. Antimicrobial activity of compounds **10** and **6** against anaerobic bacteria compared with that of erythromycin (EM) and clindamycin (CLDM).

Test organism	MIC (μg/ml) (10^6 cells/ml)			
	10	**6**	EM	CLDM
Peptostreptococcus asaccharolyticus PL-4-1	0.20	0.20	0.78	0.025
P. anaerobius GAI-1160	0.0125	0.0125	0.025	0.10
Eubacterium lentum GAI-1515	0.025	0.05	0.025	0.025
E. limosum E-531	0.0125	0.0063	0.10	0.20
Bacteroides fragilis GAI-3001	0.10	0.20	0.39	0.05
B. fragilis GAI-2552	3.1	12.5	>100	>100
B. thetaiotaomicron GAI-0659	0.20	1.56	1.56	0.025
B. ovatus Ju-26-1	0.78	1.56	6.25	0.20
B. distasonis Ju-11-1	0.10	1.56	0.78	0.20
B. vulgatus 4741	0.10	1.56	1.56	0.025
Fusobacterium nucleatum GAI-0462	0.10	0.20	6.25	0.0125
F. varium GAI-0308	25	50	100	1.6
Clostridium perfringens GAI-0084	0.20	0.78	0.39	0.0125
C. ramosum GAI-2560	0.10	0.39	0.20	0.78
C. bifermentans GAI-0209	0.025	0.025	0.10	0.20
C. sordellii GAI-0029	0.025	0.025	0.05	0.20

Medium: GAM.
EM and clindamycin were purchased from Sigma Chemical Company and their potencies were corrected with the standard samples from the National Institute of Health of Japan.

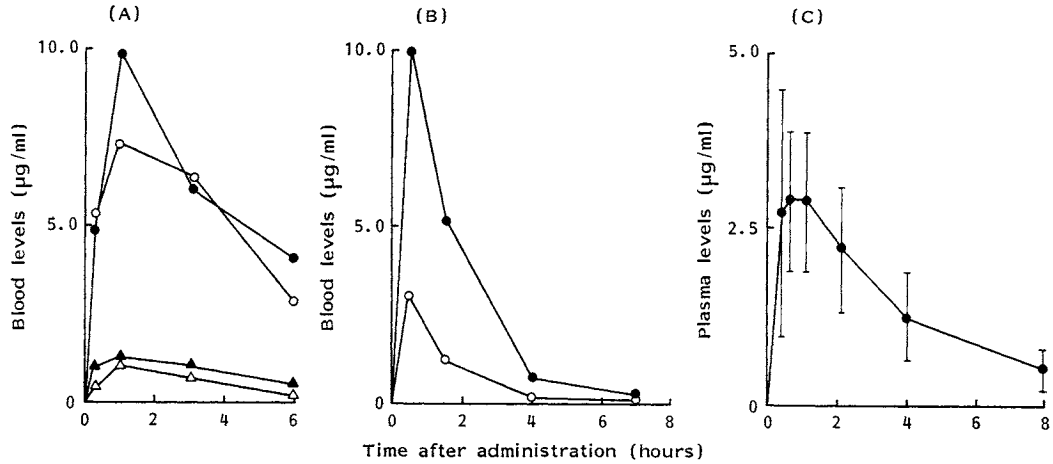
Table 5. *In vivo* activity of compounds **10** and **6** against experimental infections in mice compared with that of 4′-deoxydesmycosin (**11**) and erythromycin (EM).

Test organism	PD_{50} (mg/kg)[a]							
	10		**6**		**11**		EM	
	po	sc	po	sc	po	sc	po	sc
Staphylococcus aureus Smith	5.1	NT	43.5	NT	NT	NT	32.9	NT
Streptococcus pyogenes S-23	10.2	21.7	75.7	100	100	9.5	75.1	13.7
S. pneumoniae DP-III	65.9	30.7	100	NT	NT	5.4	20.3	6.7

[a] PD_{50}: Van der Waerden method[10]. Mice: Slc ICR (male). Challenge: 5% Mucin suspension (ip). Administration: 1 hour after challenge (sc).
NT: Not tested.

Fig. 2. Blood levels of 19-deformyl-4′-deoxydesmycosin (**10**) and other macrolides in various animals after oral administration.

(A) Mouse 100 mg/kg, (B) hamster 50 mg/kg, (C) beagle dog 10 mg/kg.
● 19-Deformyl-4′-deoxydesmycosin (**10**), ○ 19-deformyldesmycosin (**6**), ▲ 4′-deoxydesmycosin (**11**), △ erythromycin (EM).



Determination: Bioassay, paper-disk method (*Micrococcus luteus*), plotting: mean value±SE.

pound **10** also showed the best antimicrobial activity against *Mycoplasma pneumoniae* and anaerobic bacteria (Table 4). Thus, deformylation of the aldehyde function resulted in a great increase in *in vitro* activity.

In Vivo Activity

In vivo antimicrobial activity against experimental infections caused by *S. aureus* Smith, *Streptococcus pyogenes* S-23 and *Streptococcus pneumoniae* DP-III was evaluated in mice. As shown in Table 5, compound **6** was as effective as erythromycin (EM) but its 4′-deoxy derivative, 19-deformyl-4′-deoxydesmycosin (**10**), was highly effective when administered orally. The PD_{50} value[10] of compound **10** was 1/4 to 1/7 of that of EM.

These results show that deoxygenation of the 4′-hydroxyl group enhances not only *in vitro* activity but also *in vivo* activity dramatically.

Blood Levels

Compounds **10** and **6**, EM and 4′-deoxydesmycosin (**11**)[8] were given orally to laboratory animals

and their blood levels (plasma levels in beagle dogs) were compared as shown in Fig. 2. The blood levels of compounds **10** and **6** were exceedingly high in mice compared with those of compound **11** and EM. In hamsters, the blood level of compound **10** was much higher than that of compound **6**. This superior bioavailability of compound **10** was also shown in beagle dogs. The maximum concentration (Cmax) of compound **10** was 2.94 μg/ml even when administered at a 10-mg/kg dose. TLC analysis of the blood of mice showed no detectable metabolite of compound **10**, **6** or **11**.

Toxicity

Compound **10** was given intraperitoneally to Slc *dd*Y mice (male, $n=5$) at a 100-mg/kg dose to determine if it has hepatic toxicity. It was tested for subacute toxicity (14 days) in ICR JCL mice (male, $n=7$) by oral administration of a 500-mg/kg dose and a 1,000-mg/kg dose. No appreciable abnormalities were detected in the blood constituents or by pathological observation.

Conclusion

As previously reported[5], modification of the aldehyde function of desmycosin (**1**) has yielded a series of macrolide derivatives with increased oral efficacy and bioavailability. In consideration of the structure of mycinamicins, we have investigated deformylation of the aldehyde group and deoxygenation of the 4′-hydroxyl group in compound **1** and we found effective methods for these investigations as described above.

19-Deformyldesmycosin (**6**) possessed greater *in vitro* activity and *in vivo* efficacy than compound **1**. The 4′-deoxy derivative of compound **6**, 19-deformyl-4′-deoxydesmycosin (**10**), however, showed much more increased *in vivo* activity and greater bioavailability after oral administration. Thus, the deoxygenation of the C-4 hydroxyl group in mycaminose enhanced not only *in vitro* activity but also *in vivo* efficacy.

Concerning the increased *in vivo* activity caused by the conversion of the aldehyde group to a hydrophobic group, other workers suggested that the aldehyde group would be a site of rapid metabolism and its modification to a less rapidly metabolized substituent would result in greater bioavailability[5]. In the case of compound **11**, after oral administration to mice, however, no appreciable metabolites were detected, so we suggest that this greatly enhanced bioavailability of compounds **10** and **6** is caused by their increased absorption and decreased excretion rate.

These results suggest the possibility of clinical use for 19-deformyl-4′-deoxydesmycosin (**10**). Further investigation is now in progress.

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