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Syntheses of 23-Dialkylamino-23-deoxydemycinosyltylosins and Conformational Study of Mycaminosyl Tylonolide by Nuclear Overhauser Difference Spectroscopy

Shuichi SAKAMOTO, Tsutomu TSUCHIYA,* Toshiaki MIYAKE, Akihiro TANAKA, and Sumio UMEZAWA

Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki 211

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23-Dialkylamino-23-deoxydemycinosyltylosins have been prepared by treating 23-deoxy-23-iododemycinosyltylosin (**2**) with a series of amines including dimethylamine, diethylamine, and piperidine. 14(23)-Unsaturated derivatives, 23-deoxy-14(23)-enomycaminosyl tylonolide (**17**) and 23-deoxy-14(23)-enodemycinosyltylosin (**4**) have also been prepared. The conformation of mycaminosyl tylonolide dissolved in chloroform-*d* was determined by nuclear Overhauser difference spectroscopy, revealing that the two three-member groups of H(10), Me(22), and H(14) and of H(11), H(13), and H(15) stood, respectively, in a line in this order.

Demycinosyltylosin (DMT), which cannot be prepared practically by acidic hydrolysis of tylosin on account of the preferential removal of mycarose moiety from tylosin, was isolated from a culture of a blocked mutant[1] of tylosin-producing strain, *Streptomyces fradiae*. Okamoto *et al.*[2] also prepared the same compound in large amounts by fermentation of a blocked mutant (YO-9010) of *Streptomyces fradiae* NRRL 2702. Tatsuta *et al.*[3] synthesized the compound in the course of the total synthesis of tylosin. The compound has a reactive primary hydroxyl group at C-23, and this stimulated us to prepare derivatives having functional groups other than the hydroxyl at the C-23.

Recently we prepared 23-dialkylamino derivatives[4] of mycaminosyl tylonolide (MT) and 4′-deoxymycaminosyl tylonolide[5] (DT) by converting their hydroxyls at C-23 to dialkylamino groups *via* the corresponding iodo derivatives and found that the dialkylamino derivatives showed strong antibacterial activities against Gram-negative bacteria. Since DMT (**1**) includes MT-mycarose (1″→4′) structure, the C-23-dialkylamino derivatives of **1** were expected to have similar antibacterial activities as well.

Results and Discussion

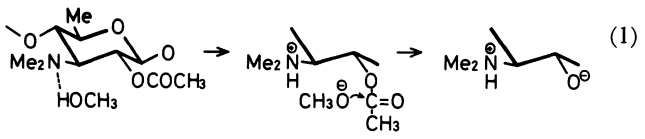
Treatment of DMT (**1**) with iodine and triphenylphosphine according to Matsubara *et al.*[6] but changing the solvent to pyridine,[7] without protection of the formyl group, gave readily 23-deoxy-23-iododemycinosyltylosin (**2**). The structure was confirmed by the ^{1}H-NMR spectrum. Treatment of the iodo derivative (**2**) with dimethylamine in hot acetonitrile according to Tanaka *et al.*[4] gave the 23-deoxy-23-dimethylamino derivative (**3**) in a short period in high yield (83%) with slight 14(23)-ene product (**4**) accompanied. If, in this reaction, excess dimethylamine was used, proportion of **4** increased. In a similar fashion, other 23-deoxy-23-dialkylamino derivatives of **1** were prepared from **2** and a series of dialkylamines, namely, diethylamine, *N*-methyl-2-hydroxyethylamine, pyrrolidine, piperidine, hexahydroazepine, and octahydroazocine (giving **9**—**14**, respectively). Reaction conditions and analytical data of these products are shown in Table 1.

4″-*O*-Acetyl derivative (**7**) of **3** was next prepared. Treatment of **3** with acetic anhydride in acetonitrile in the presence of sodium hydrogencarbonate at room temperature gave almost quantitatively the 2′-*O*-acetyl derivative (**5**), which was then acetylated with acetic anhydride in pyridine under conditions to avoid the acetylation of HO-3. After a week at 0°C, 2′,4″-di-*O*-acetyl derivative (**6**) was obtained in a moderate yield (52%) with the starting material (**5**) and 2′,3,4″-tri-*O*-acetyl compound (**8**). Compound **8** was prepared, in a separate experiment, by per-*O*-acetylation of **3** (see Experimental). Heating **6** in methanol selectively cleaved the 2′-*O*-acetyl group to give the 4″-*O*-acetyl derivative (**7**) in high yield. Possibly, the selective deacetylation at C-2′ may be due to the presence of the dimethylamino group at C-3′ which attracts a proton

TABLE 1. REACTION PERIODS, YIELDS FROM **2**, OPTICAL ROTATIONS AND ELEMENTAL ANALYSIS OF COMPOUNDS **3** AND **9**—**14**

Compound formed	Reaction period (min)	Yield %	$[\alpha]_D^{22}$ (c 1, $CHCl_3$)	Formula	C Calcd	C Found	H Calcd	H Found	N Calcd	N Found
3	60	83	−26°	$C_{40}H_{70}N_2O_{12}\cdot 1/2H_2O$	61.78	61.82	8.88	8.60	3.60	3.79
9	200	61	−23°	$C_{42}H_{72}N_2O_{12}$	63.32	63.00	9.05	8.85	3.52	3.57
10	150	79	−19°	$C_{41}H_{70}N_2O_{13}\cdot H_2O$	60.29	60.24	8.82	8.50	3.43	3.47
11	60	49	−24°	$C_{42}H_{70}N_2O_{12}\cdot H_2O$	62.07	62.38	8.87	8.53	3.45	3.73
12	75	76	−17°	$C_{43}H_{72}N_2O_{12}\cdot H_2O$	62.47	62.62	8.96	8.70	3.39	3.04
13	60	62	−14°	$C_{44}H_{74}N_2O_{12}\cdot H_2O$	62.86	62.87	9.05	8.72	3.33	3.19
14	60	60	−15°	$C_{45}H_{76}N_2O_{12}\cdot H_2O$	63.23	63.12	9.13	9.30	3.28	3.27

from a methanol molecule to form an ion pair as depicted in I, followed by attack of the MeO^- upon the acetyl carbon as shown in Formula 1.



(1)

I

^{1}H-NMR Spectral Studies. Chemical structures of **2**, **3**, **5**, **6**, **7**, and **8** were confirmed by their ^{1}H-NMR spectra. The proton chemical shifts of these compounds, MT, and DMT (**1**) are shown in Table 2, and the spectrum of MT, in Fig. 1. In MT and DMT, no substantial differences in shift values between the corresponding protons of both compounds were observed, although slight differences being observed in H-1′, 2′, 3′, 4′, and *N*-methyls at C-3′. In the 23-iodo compound (**2**), pronounced up-field shifts of methylene protons at C-23 were observed, in comparison to **1**, and moderate shift changes in H-13, 14, and 15, all of these hydrogen atoms locating in the vicinity of C-23. In the 23-dimethylamino derivative (**3**), pronounced up-field shifts of the methylene protons at C-23 were observed, in comparison to the iodo derivative (**2**), and moderate to slight shift changes in H-10, 13, 14, and 15. These

Table 2. Proton chemical shift data[a] of MT, DMT(**1**), **2**, **3**, **4**, **5**, **6**, **7**, and **8** (in $CDCl_3$ at 50°[b] for MT and DMT, and 24° for other compounds)

H-atom	MT	DMT(1)	2	3	5	6	7	8	4
2a	1.97 d	1.95	1.93	1.94	1.91	1.91	1.94	1.97	
2b	2.50 dd	2.51	2.53	2.52	2.49	2.48	2.51	2.59	
3	3.87 d[c]	3.85[c]	3.84[c]	3.84[c]	3.82[c]	3.82[c]	3.84[c]	5.12	3.85 d[c]
4	≈1.64 m	≈1.64	≈1.64						
5	3.75 ddd	≈3.74	3.72	3.72 **	3.61	3.62 **	3.72	3.45	3.75
6	2.23 m[d]	2.20[d]	2.18[d]						
7a	1.48 d	1.49	1.50	≈1.5		≈1.5			
7b	≈1.64 m	≈1.64	≈1.64						
8	2.68 m[d]	* 2.64	≈2.62	≈2.6	* ≈2.52	≈2.52	* ≈2.57	2.58	
10	6.27 d	6.29 *	6.33 *	6.25	6.27	6.27	6.24	6.27	6.32 d
11	7.30 d	7.31	7.33	7.36	7.36	7.34	7.37	7.43	7.21 d
13	5.88 d[c]	5.86[c] **	5.71[c] *	5.76[c]	5.76[c]	5.74[c]	5.76[c]	5.82	6.20 s
14	2.88 ddd	≈2.91 m **	2.81 dq *	≈2.87 m	≈2.86	≈2.84	≈2.85	≈2.87 m	
15	4.98 dt	4.97 **	4.86 **	4.75	4.74	4.77	4.75	4.63	5.38 t
16a	≈1.68 m	≈1.65	≈1.68						≈1.76
16b	1.86 m	≈1.88							≈1.90
17	0.95 t	0.95	0.96	0.93	0.94	0.94	0.94	0.92	0.99
18	1.01 d	1.00	1.01	1.01	* 0.93	0.92	* 1.01	0.97	0.98
19a	2.36 dd	2.39							
19b	2.91 dd	* ≈2.85				≈2.87	≈2.85		
20	9.69 s	9.68	9.69	9.69	9.67 d[e]	9.68[e]	9.68 s	9.61 d[e]	9.74 s
21	1.22 d	1.21	1.23	1.22	1.22	1.22	1.20	1.23	1.20
22	1.83 d[e]	1.83[e]	1.83[e]	1.83[e]	1.83[e]	1.82[e]	1.83[e]	1.83[e]	1.86[e]
23a	≈3.73	≈3.74 ***	3.10 dd ***	2.29	2.29	2.31	2.28	2.29	5.09 s
23b		***	3.27 ***	2.53 dd *	2.47	2.47	2.46	2.46	5.33 s
C(23)NMe_2				2.19 s	2.19	2.19	2.19	2.19	
1′	4.30 d	* 4.23	4.22	4.22 *	4.27	4.29 *	4.22	4.25	4.25
2′	3.49 dd	* 3.54	3.54	3.54 ***	5.01	5.01 ***	3.52	5.01	3.57
3′	2.43 t	* 2.47		≈2.47 ***	2.69	2.71 ***	≈2.45	2.70	
4′	3.09 t	**			3.29	3.29	≈3.28		
5′	3.31 m	* 3.21—3.33	3.2—3.33	3.2—3.33 **	* 3.24	3.26	≈3.28	3.23—3.25	
6′	1.28 d	1.26[f]	1.25[f]	1.24[f]	1.26[f]	1.26	1.24	1.26[f]	1.27
C(3′)NMe_2	2.55 s	* 2.49	2.50	2.49 **	2.39	2.42 **	2.52	2.41	2.50
1″		5.06 d	5.08	5.08	5.07	5.06	5.07	5.07	5.09
2″a		1.76 dd	1.76	1.77	1.77 *	1.84	1.85	1.85	1.77
2″b		2.03 d	2.03	2.02	2.03 *	1.98 *	2.02	1.99	2.04
C(3″)Me		1.33 s *	1.24	1.24	1.23 **	1.11	1.13	1.13	1.24
4″		≈2.91			≈2.90 ***	4.60 d	4.62	4.61	
5″		4.07 dq	4.06	4.06	* 4.01 ***	4.29 **	4.46	4.53	4.08 m
6″		1.29 d	1.30	1.30	1.29 **	1.13	1.14	1.14	1.30
3-OAc								2.11	
2′-OAc					2.08 s	2.06		2.07	
4″-OAc						2.14 s	2.16	2.16	

a) After the shift values (δ), multiplicity of the signals is cited, that is omitted if the multiplicity is the same with that of the left-handed compound. Asterisk, *, **, and *** mean that the $\Delta\delta$ between the figures of the right and the left are 0.04—0.09, 0.1—0.2, and >0.2ppm, respectively, except for **4** and **8**. b) Signals of MT and DMT were not sharp at 24°. c) Slightly broadened, d) Broadened, e) $J \leq 1$ Hz, f) Signal height is 60—70% of that of H-6″.

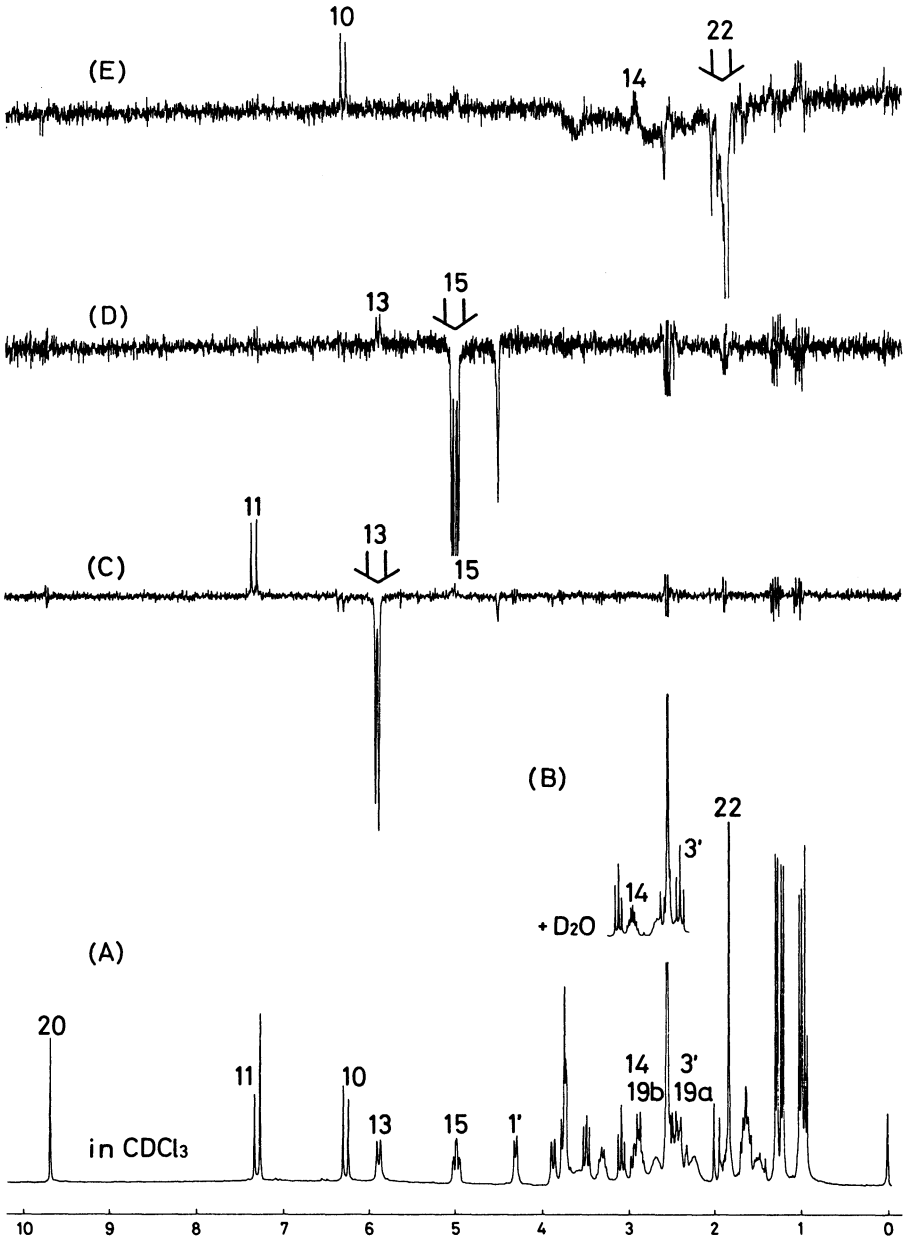


Fig. 1. ^{1}H-NMR spectra of MT. (A) Normal spectrum, (B) measured after addition of D_2O followed by standing for 5d with occasional shaking (indicating that H-19a, b were deuterated), (C–E) NOE difference spectrum; arrows mean the positions irradiated.

results indicate that functional group changes occur at the C-23. The momo-*O*-acetyl derivative (**5**) of **3** showed pronounced down-field shift of H-2′, and moderate shift changes of *N*-methyls at C-3′, H-4′, and 5′, in comparison to the corresponding chemical shifts of **3**, indicating that HO-2′ was acetylated. The di-*O*-acetyl derivative (**6**) showed pronounced down-field shifts of H-2′ and 4″ in comparison to the corresponding shifts of **3**, indicating that HO-4″ (and not HO-3) was acetylated in addition to HO-2′. Selective deacetylation of **6** gave monoacetyl derivative (**7**), that showed up-field shift of H-2′, indicating that 2′-*O*-acetyl group was removed. It is worthy to mention that *O*-acetyl methyl protons at C-2′, 3, and 4″ resonated invariably at δ 2.08, 2.11, and 2.16, respectively in the four compounds (**5**, **6**, **7**, **8**) usable for diagnosis of the acetyl positions.

Decoupling results obtained in MT are shown in Table 3, with the general coupling constants relating to **2**, **3**, **5**, **6**, **7**, **8**, MT, and DMT. As was cited in the Table, substantially the same J values were observed in the corresponding protons in all compounds described above, with slight exceptions, and the values showed good accordance with those of rosaramicin reported.[8]

Some stereochemical problems between the range of C-10 and C-15 are next described. The large coupling constants of $J_{10,11}$ in the compounds described above indicate that H-10 and 11 are in trans relationship, as repeatedly reported in similar compounds. However, ^{1}H-NMR spectral study in a solution describing the

TABLE 3. DECOUPLING DATA OF MT (UNLESS OTHERWISE STATED) AND COUPLING CONSTANTS RELATING TO MT, DMT(**1**), **2**, **3**, **5**, **6**, **7**, AND **8**

Position to be irradiated (δ)	Observed[a)]	A	B	$J_{A,B}$(Hz)
2.50 (H-2b, partly H-3′)	2a→s, 3→s, 2′→incomplete t,	2a,	2b	17
	4′→change	2a,	3	0
		2b,	3	10—10.5
3.87 (H-3)	2b→d (*J* 17), 2a→no change	3,	4	≈1
		4,	5	9.3—10.5
2.23 (H-6)	5→no change,	5,	6	0
	7a→change, 7b→change,	6,	19a	4.5
	19a, 19b→ABq	6,	19b	10
		19a,	19b	≈17
1.48 (H-7a)	6 and 8→not clear	6,	7a	10.5
		6,	7b	
2.68 (H-8)	7a→dd (*J* 10, 15), 7b→change,	7a,	7b	15
	21→s	7a,	8	5
		7b,	8	≈12
1.22 (H-21)	8→simplified	8,	21	6.5
1.21 (H-21) DMT	8→simplified			
7.30 (H-11)	10→s	10,	11	15.5—16
5.88 (H-13)	14→dq, 22→s	13,	22	≈1
2.90 (H-14,19b)	6→slight change, 13→s,	13,	14	10.5
	15→dd (*J* 3, 10), 19a→br. s,	14,	23a	4.5 (for **3**, **5**, **6**)
	23a,b→br. s,	14,	23b	10 (for **3**, **5**, **6**)
		23a,	23b	12 (for **3**, **5**, **6**)
		14,	15	9.5
3.74 (H-5, 23a,b)	6→change, 14→t (*J* 9.5)			
1.61 (H-4, 7b,16a)	3→no change, 8→change	15,	16a	9.5
	15→change, 17→change,	15,	16b	3
	18→s	16a,b,	17	6—7.5
1.68 (H-16a)	5→incomplete s, 15→d (*J* 9),	18,	4	6—7
	17→slight change, 18→incomplete s			
4.98 (H-15)	16a→q, 16b→q, 14→change			
1.86 (H-16b)	15→t (*J* 9.5), 17→d			
3.09 (H-4′)	3′→d (*J* 10)	1′,	2′	7.5
3.27 (H-4′, 5′) DMT	6′→s, 3′→not clear	2′,	3′	10—10.5
		3′,	4′	10
		4′,	5′	9.3
		5′,	6′	6—6.3
5.06 (H-1″) DMT	2″a→d (*J* 15)	1″,	2″a	3.5—4
1.76 (H-2″a) DMT	1″→s, 2″b→s	1″,	2″b	0
2.91 (H-4″) DMT	5″→q	2″a,	2″b	14—15
4.07 (H-5″) DMT	6″→s	4″,	5″	9.5—10
		5″,	6″	6.3—6.5

a) The figures after the arrow mean the multiplicity after irradiation.

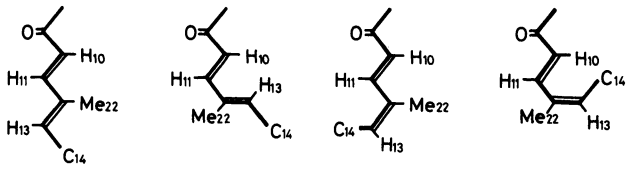


Chart 1.

spacial situation related to H-11, Me-22, and H-13 has not been reported yet. Theoretically, the following four structures (I, II, III, and IV) should be considered. Omura *et al.*[9] reported, by the X-ray crystallographic study of protylonolide, that Me-22 and H-13 are trans, and that Me-22 and H-11 are also trans. From the former result, structures III and IV should be abandoned. However, in the latter relationship, possibility of structure II should be considered, because, in a solution, rotational energy around the axis of C(11)—C(12), is thought to be not so high as to prevent the debate. To clarify the problem, nuclear Overhauser effects (NOE) between some important hydrogen atoms were measured using the NOE difference spectroscopy[10] (Fig. 1). Irradiation at δ 1.83 (Me-22) of MT caused pronounced positive enhancement (≈15%) of each of the resonances of H-10* and 14, but not other resonances including H-11* and 13. On this experiment, if strongly irradiated, slight enhancement of H-15 was observed. This may be caused by the simultaneous irradiation of H-16b (δ ≈1.86) as well as

*Resonances at δ 6.3 and 7.3, both appeared as a doublet, were assigned to H-10 and 11, respectively. These assignments will be reasonable from the point of view of mesomeric effect[11a)] and the comparison of the chemical shifts of the corresponding protons of, for example, mycinamicins,[12)] which have a similar α,β,γ,δ-unsaturated carbonyl fragments lacking the methyl group at C-12.

Me-22, and H-15 may be enhanced through space or through bond interaction. Irradiation of H-11 and H-13 also caused pronounced positive enhancements (20—22%) of H-13 and H-11, respectively, with small signal increase (5—6%) of H-15 in the latter case. Irradiation of H-15 also caused enhancement (≈5%) of H-13. In conclusion, these results show that Me-22 is located in close proximity with both H-10 and 14, that is, between H-10 and 14, and H-13 is located be-

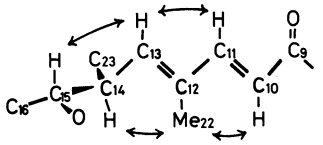


Chart 2.

tween H-11 and 15, as depicted above. The conclusion clearly supported the structure of I having *s*-trans[13] structure, and also confirmed the absolute configuration at C-15 to be *R*.

The structure of **4** was confirmed by the ^{1}H-NMR spectrum (Table 2). In the spectrum, H-13 appeared as a singlet suggesting the absence of H-14. Resonances of methylene protons at C-23, which appeared at $\delta \approx 3.7$ in MT, appeared as two singlets at δ 5.09 and 5.33. The chemical shifts and zero coupling suggest[11b] the presence of a terminal double bond.

To confirm the structure of **4** further, 23-deoxy-14(23)-enomycaminosyl tylonolide (**17**), a simpler analog, was prepared starting from 23-bromo-23-deoxymycaminosyl tylonolide diethyl acetal[7] (**15**) with silver fluoride *via* the corresponding 14(23)-ene compound (**16**). The ^{1}H-NMR spectra of the macrolactone portions of **17** and **4** showed good accordance.

Antibacterial spectra (Table 4) of the new derivatives showed that introduction of a dialkylamino group at C-23 of DMT (**1**) gave activities, comparable to that of parent DMT. Among the compounds, the 4″-*O*-acetyl derivative (**7**), 23-deoxy-23-piperidino and hexahydroazepine-1-yl derivatives (**12**, **13**) were most active against both Gram-positive and-negative bacteria.

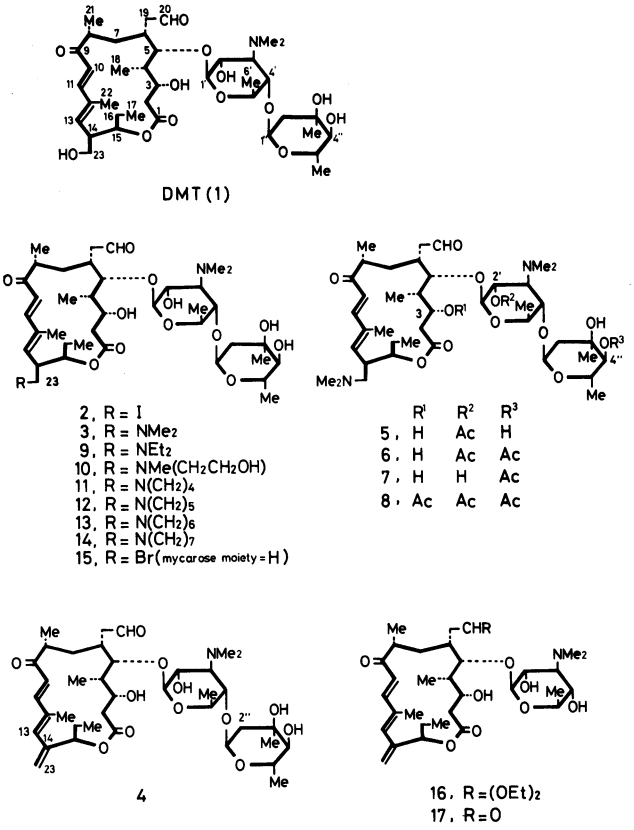


Chart 3.

Experimental

General. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Thin-layer chromatography (TLC) was carried out on Kieselgel 60 F-254 (E. Merck) silica gel with detection by spraying with sulfuric acid, followed by slight heating. Column chromatography was performed on Wakogel C-200, or Kieselgel 60, 230—400 mesh (E. Merck). UV spectrum was recorded with a Hitachi 200-10 spectrometer. ^{1}H-NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer, or at 250 MHz in the FT mode with a Bruker WM 250 spectrometer at 24°C unless otherwise stated.

23-Deoxy-23-iododemycinosyltylosin (2). A mixture of crude demycinosyltylosin (**1**, 1.06 g) obtained from a culture, triphenylphosphine (0.93 g), and iodine (0.4 g) in dry pyridine (52 ml) was stirred under nitrogen at 0°C for 3.5 h. After addition of 0.1 M aqueous sodium thiosulfate (3 ml) to decompose the excess iodine, the mixture was evaporated. The chloroform solution of the residue was washed with saturated aqueous sodium hydrogencarbonate, saturated aqueous sodium sulfate, dried (Na_2SO_4), and evaporated. Silica-gel column chromatography of the residue with chloroform–methanol–28% aqueous ammonia (50:1:0.1) as the eluant gave a solid of **2**, 930 mg. It showed, on TLC with chloroform–methanol–28% aqueous ammonia (10:1:0.1), a single

TABLE 4. MINIMAL INHIBITORY CONCENTRATIONS (μg/ml) OF THE PRODUCTS

	3	**7**	**9**	**10**	**11**	**12**	**13**	**14**	DMT
Staph. aureus 209P	0.78	0.78	0.78	1.56	1.56	0.78	0.78	0.78	0.78
Sarcina lutea PCI 1001	0.39	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
B. subtilis B-558	0.39	0.78	0.78	0.78	0.78	0.39	0.39	0.78	0.78
E. coli NIHJ	3.12	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
Kl. pneumoniae PCI 602	6.25	3.12	6.25	6.25	6.25	3.12	3.12	3.12	6.25
Sh. dysenteriae JS 11910	6.25	3.12	6.25	1.56	1.56	1.56	1.56	3.12	1.56
Sal. enteritidis 1891	3.12	6.25	6.25	3.12	6.25	3.12	3.12	3.12	6.25

spot of R_f 0.47 (*cf* **1**, 0.32), $[\alpha]_D^{22}$+4° (*c* 1, chloroform). ^{1}H-NMR ($CDCl_3$): $J_{14,23a}$ 9, $J_{14,23b}$≈3, $J_{23a,23b}$ 10 Hz. Irradiation of H-14 collapsed the signals of H-15, H-23a, 23b, and H-13 to double doublets, a doublet, a doublet, and a singlet, respectively. Irradiation at δ 3.3 (H-23b), or of H-13 collapsed the signals of H-14 to a quartet, and double triplets, respectively. Irradiation of H-1″ collapsed the double doublets of H-2″a to a doublet.

Found: C, 52.76; H, 7.05; N, 1.36; I, 14.52%. Calcd for $C_{38}H_{62}INO_{12}\cdot H_2O$: C, 52.47; H, 7.36; N, 1.61; I, 14.61%.

23-Deoxy-23-dimethylaminodemycinosyltylosin (3) and 23-Deoxy-14(23)-enodemycinosyltylosin (4). A solution of **2** (750mg) and dimethylamine (0.88ml of 5M solution in acetonitrile (1M=1 mol dm^{-3}); 5mol equivalents for **2**) in acetonitrile (15ml) was heated at 80°C for 30min. The same amount of dimethylamine was added, and the reaction was continued for further 30min. On TLC with chloroform–methanol–28% aqueous ammonia (10:1:0.1), the solution showed spots of R_f 0.35 (**3**) and 0.47 (slight, **4**, the R_f value being the same with that of **2**). Evaporation gave a residue, which was extracted with chloroform. The organic layer was washed with saturated aqueous sodium hydrogencarbonate and saturated aqueous sodium sulfate, dried (Na_2SO_4), and evaporated to give a solid (718mg). Purification by silica-gel column chromatography with chloroform–methanol–28% aqueous ammonia (40:1:0.1) gave solids of **3**, 564mg (83%) and **4**, 71mg (11%).

Compound **4**: $[\alpha]_D^{20}$+11° (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $J_{2b,3}$ 10, $J_{4,5}$ 7, $J_{10,11}$ 16, $J_{13,22}$ 1, $J_{15,16a}$=$J_{15,16b}$ ≈6.5, $J_{23a,23b}$ 0, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10, $J_{1'',2''a}$ 3.7, $J_{1'',2''b}$ 0, $J_{2''a,2''b}$ 15Hz.

Irradiation at δ 1.83 (H-16a,b) collapsed the triplet of H-15 and H-17 to a singlet, respectively. Irradiation of H-13 collapsed the small doublet of H-22 to a singlet. Irradiation of H-1″ collapsed the double doublets of H-2″a to a doublet.

Found: C, 59.74; H, 7.91; N, 1.78%. Calcd for $C_{38}H_{61}NO_{12}\cdot H_2CO_3$: C, 59.62; H, 8.03; N, 1.78%.

2′-O-Acetyl-23-deoxy-23-dimethylaminodemycinosyltylosin (5). To a solution of **3** (564mg) in acetonitrile (11ml) were added acetic anhydride (0.37ml) and powdered sodium hydrogencarbonate (0.6g) and the mixture was stirred at room temperature for 4h. Evaporation gave a residue, which was treated with ice-cold aqueous half-saturated sodium hydrogencarbonate under stirring for 30min. Extraction of the mixture with chloroform, washing the organic layer with a saturated aqueous sodium sulfate, drying over sodium sulfate, and evaporation gave a solid of **5**, 568mg (95%), $[\alpha]_D^{20}$−37° (*c* 1, chloroform).

Found: C, 62.05; H, 8.58; N, 3.36%. Calcd for $C_{42}H_{70}N_2O_{13}$; C, 62.22; H, 8.64; H, 3.46%.

2′,4″-Di-O-acetyl-23-deoxy-23-dimethylaminodemycinosyltylosin (6). A mixture of **5** (43.6mg) and acetic anhydride (8.2μl) in pyridine (0.22ml) was kept at 0°C for 7d. After addition of water (0.05ml), followed by standing for a few min, the mixture was poured into saturated aqueous sodium hydrogencarbonate (2ml). Extraction of the mixture with chloroform, washing the organic layer with saturated aqueous sodium hydrogencarbonate and then saturated aqueous sodium sulfate, drying over sodium sulfate, and evaporation gave a residue. Silica-gel column chromatography of the residue with toluene–acetone (2:1) as the developer gave **6**, 24mg (52%) and a mixture of **5** recovered and tri-*O*-acetyl compound, 19.7mg. **6**: $[\alpha]_D^{20}$−47° (*c* 1, chloroform).

Found: C, 61.68; H, 8.38; N, 3.12%. Calcd for $C_{44}H_{72}N_2O_{14}$: C, 61.97; H, 8.45; N, 3.29%.

4″-O-Acetyl-23-deoxy-23-dimethylaminodemycinosyltylosin (7). A solution of **6** (24mg) in methanol (0.24ml) was heated at 60°C overnight. Evaporation gave a solid, that was chromatographed on a silica-gel column with chloroform–methanol–28% aqueous ammonia (50:1:0.1) to give a solid of **7**, 20.9mg (91.5%), $[\alpha]_D^{22}$−28° (*c* 1, chloroform).

Found: C, 60.56; H, 8.17; N, 3.26%. Calcd for $C_{42}H_{70}N_2O_{13}\cdot 1/2H_2CO_3$: C, 60.64; H, 8.44; N, 3.33%.

2′,3,4″-Tri-O-acetyl-23-deoxy-23-dimethylaminodemycinosyltylosin (8). A mixture of **3** (21mg) and acetic anhydride (42μl) in pyridine (0.1ml) was kept at 0°C for 2d. After addition of water (20μl) the solution was concentrated. The chloroform solution of the residue was treated as usual to give, after chromatography, a solid of **8**, 15mg (63%), $[\alpha]_D^{20}$−35° (*c* 1, chloroform).

^{1}H-NMR ($CDCl_3$): Irradiation of H-2b collapsed the doublet of H-2a and the broad doublet of H-3 to a singlet, respectively. Irradiation at δ 1.80 (H-2″a) collapsed the doublet of H-1″ to a singlet.

Found: C, 60.95; H, 8.07; N, 3.06%. Calcd for $C_{46}H_{74}N_2O_{15}\cdot 1/2H_2O$: C, 61.13; H, 8.31; N, 3.10%.

Preparation of 9, 10, 11, 12, 13, and 14. To a solution of **2** (≈100mg) in acetonitrile (2ml) was added the corresponding amine (5 equivalents for **2**), that is, diethylamine (for the preparation of **9**), *N*-methyl-2-hydroxyethylamine (for **10**), pyrrolidine (for **11**), piperidine (for **12**), hexahydroazepine (for **13**), and octahydroazocine (for **14**), and the solution was heated at 80°C for the period cited in Table 1. Work-up as described for **3** gave the desired 23-dialkylamino derivatives.

^{1}H-NMR spectra of **9**, **10**, **11**, **12**, **13**, and **14** in $CDCl_3$. Compound **9**: H-23a signals did not appear near δ 2.3, and were hardly discernible by overlapping by other signals. Some other signals were: δ 4.75 (1H, dt, H-15), 5.76 (1H, d, H-13), 6.25 (1H, d, H-10).

Compound **10**: δ 1.83 (1H, d, H-22), 2.24 (3H, s, NMe), 4.76 (1H, dt, H-15), 5.74 (1H, d, H-13), 6.28 (1H, d, H-10).

Compound **11**: δ 2.54 (1H, dd, *J* 4.5 and 12Hz, H-23b), 2.63 (1H, dd, *J* 10 and 12Hz, H-23a), 4.77 (1H, dt, H-15), 5.80 (1H, d, H-13), 6.26 (1H, d, H-10).

Compound **12**: δ 4.75 (H-15), 5.76 (H-13), 6.26 (H-10).

Compound **13**: δ ≈2.57 (H-23a) 2.66 (1H, dd, *J* 4.5 and 12Hz, H-23b), 4.77 (H-15), 5.79 (H-13), 6.26 (H-10).

Compound **14**: δ 4.75 (H-15), 5.81 (H-13), 6.26 (H-10).

23-Deoxy-14(23)-enomycaminosyl Tylonolide Diethyl Acetal (16). To a solution of **15**[7] (55mg) in pyridine (0.55ml) was added silver fluoride (30mg) and the mixture was stirred at room temperature overnight. The solution showed, on TLC with chloroform–methanol (6:1), a single spot having the same R_f value with that of **15**. Concentration gave a residue, which was extracted with dichloromethane. Evaporation with several additions of toluene gave a solid, which was purified by column chromatography with chloroform–methanol (6:1) to give a solid, 47.1mg (96%), $[\alpha]_D^{25}$+19° (*c* 1, chloroform) ^{1}H-NMR ($CDCl_3$): δ 1.90 (3H, d, H-22), 2.55 (6H, s, NMe_2), 4.35 (1H, d, $J_{1',2'}$ 7.5Hz, H-1′), 5.12 (1H, s, H-23a), 5.35 (1H, s, H-23b), ≈5.3 (1H, H-15), 6.22 (1H, s, H-13), 6.42 (1H, d, $J_{10,11}$ 16Hz, H-10), 7.22 (1H, d, H-11).

Found: C, 64.24; H, 8.93; N, 2.13%. Calcd for $C_{35}H_{59}NO_{10}$: C, 64.29; H, 9.10; N, 2.14%.

23-Deoxy-14(23)enomycaminosyl Tylonolide (17). A solution of **16** (30mg) in a mixture of acetonitrile (0.6ml) and 0.1M aqueous hydrochloric acid (0.92ml) was kept at

room temperature for 1 h. The solution showed, on TLC with chloroform–methanol (6:1), a spot at R_f 0.23 (*cf* **16**: R_f 0.32). Neutralization with sodium hydrogencarbonate followed by usual work-up gave a solid, which was purified by column chromatography with chloroform–methanol–28% aqueous ammonia (13:1:0.1) to give a solid, 21.4 mg (88%), $[\alpha]_D^{25}$ +59° (*c* 1, chloroform), λ_{max}^{MeOH} 292 (ε 23000). This value was in good agreement with the calculated value (293) by Woodward generalization[14], treating the unsaturated ketone as an acylic system.

^{1}H-NMR ($CDCl_3$): δ 1.90 (3H, d, H-22), 2.53 (6H, s, NMe_2), 4.33 (1H, d, $J_{1',2'}$ 7.5 Hz), 5.13 (1H, s, H-23a), 5.37 (1H, s, H-23b), 6.26 (1H, s, H-13), 6.38 (1H, d, $J_{10,11}$ 16 Hz, H-16), 7.28 (1H, d, H-11), 9.88 (1H, s, H-20).

Found: C, 64.49; H, 8.68; N, 2.34%. Calcd for $C_{31}H_{49}NO_9$: C, 64.23; H, 8.52; N, 2.42%.

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