December, 1981] © *1981 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, *54*, *3837—3845 (1981)* 3837

Syntheses of Recyclized Macrolide Antibiotics and Related Derivatives from Mycaminosyltylonolide

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(Received June 30, 1981)

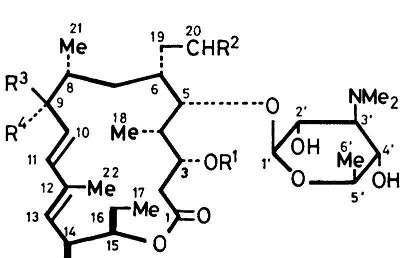
Mycaminosyltylonolide diethyl acetal was, after reduction of the 9-keto group, hydrolyzed, and the seco-acids obtained were recyclized to give new 16-membered (9*R*)- and (9*S*)-hydroxy macrolide derivatives, in which the 23-hydroxyl group of the starting substance was incorporated into the macrolactone ring. Selective oxidation of the (9*S*)-hydroxy derivative (**15**) with DDQ gave the keto compound (**18**). Removal of the acetal-protecting groups gave a new macrolide (**19**), which is antibacterial. Several other *O*-acetyl derivatives were also prepared and their antibacterial activities were measured. By the ^{1}H- and ^{13}C-NMR spectroscopies of 3,23-di-*O*-acetylmycaminosyltylonolide diethyl acetal by varying the temperature, the presence of two rotamers was clarified.

Mycaminosyltylonolide[1] (**1**) easily obtained by acidic hydrolysis of tylosin,[2] a typical 16-membered macrolide antibiotic, has fairly strong antibacterial activity, inspite of the relatively simple structure. Since structurally related demycarosyljosamycin[3] obtained by acidic hydrolysis of josamycin,[4] and demycarosylcarbomycin B,[5] both having mycaminosylmacrolactone structure, have much weaker antibacterial activity, the activity of **1** will partially be ascribable to the structure of the macrolactone portion, tylonolide. In this paper, we describe a new mycaminosylmacrolactone (**19**) obtained by recyclizing the 23-hydroxyl group of the seco-acid of **1** to the carboxylic acid to form a 16-membered lactone.

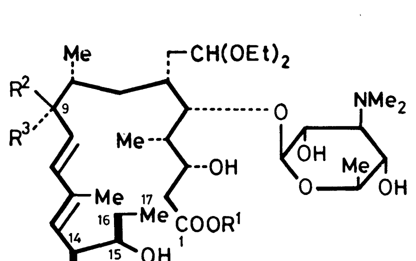
Synthesis of the new mycaminosylmacrolactone was started from mycaminosyltylonolide diethyl acetal (**2**) prepared from **1**. To protect the C-8 position from epimerization during the synthesis, the carbonyl at C-9 of **2** was reduced with sodium borohydride following the procedure by Masamune *et al.*,[6] to give two 9-hydroxy derivatives. The configurations at C-9 of the faster- (**6**) and the slower-moving derivatives (**7**) were decided from the studies of their ^{1}H-NMR spectra. Since structurally related macrolide, josamycin (leucomycin A_3) (9*R* epimer) and 9-epi-josamycin (9*S* epimer) show, in their ^{1}H-NMR spectra,[7] the coupling constants of $J_{9,10}$ 9.0 and 4.0 Hz, respectively, **6** ($J_{9,10}$ 8.0 Hz) and **7** ($J_{9,10}$ 4.5 Hz) could reasonably be assigned as 9*R*- and 9*S*-isomers. Removal of the acetal protecting groups of **6** and **7** by treatment in acidic aqueous acetonitrile gave the corresponding aldehyde derivatives (**8** and **9**) in high yields. Again the $J_{9,10}$ of **8** and **9** were shown to be 8 and 4 Hz, respectively. The deblocking of the acetal-protecting groups is necessary in order to have antibacterial derivatives.

Ring-opening of the macrolactone portion of the mixture of **6** and **7** to 15-hydroxy carboxylic acids (**10, 11**) (seco-acids) was performed by treating the mixture with sodium hydroxide in aqueous methanol. The completion of the opening was verified by the lack, in the ^{1}H-NMR spectrum, of the H-15 proton signals near δ 4—5. The structures were also confirmed by the formation of the corresponding esters (**12, 13**).

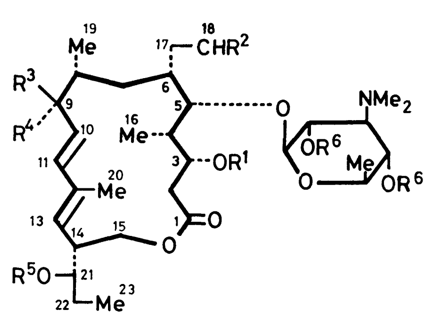
Recyclization of **10** and **11** was carried out by applying the double activation method developed by Corey *et al.*[8] Treatment of the mixture (**10, 11**) with 2,2′-dipyridyl disulfide and triphenylphosphine according to Mukaiyama *et al.*[9] gave intermediary active esters,



	R¹	R²	R³	R⁴	R⁵
1	H	=O	=O		H
2	H	(OEt)₂	=O		H
3	Ac	(OEt)₂	=O		Ac
4	Ac	=O	=O		Ac
5	Ac	=O	=O		H
6	H	(OEt)₂	H	OH	H
7	H	(OEt)₂	OH	H	H
8	H	=O	H	OH	H
9	H	=O	OH	H	H



	R¹	R²	R³
10	H	H	OH
11	H	OH	H
12, 13	Me	H	OH
	Me	OH	H



	R¹	R²	R³	R⁴	R⁵	R⁶
14	H	(OEt)₂	H	OH	H	H
15	H	(OEt)₂	OH	H	H	H
16	H	=O	H	OH	H	H
17	H	=O	OH	H	H	H
18	H	(OEt)₂	=O		H	H
19	H	=O	=O		H	H
20	Ac	(OEt)₂	=O		Ac	Ac
21	Ac	(OEt)₂	=O		Ac	H
22	Ac	=O	=O		Ac	H

which were then diluted (0.003—0.006 M) with toluene and heated (80 °C, 24 h) to give a mixture of recyclized products (**14** and **15**) in 17% yield (after purification). Similar treatment of the acids (**10**, **11**) with bis(4-*t*-butyl-1-isopropyl-2-imidazolyl)disulfide[10] and triphenylphosphine followed by dilution with toluene and heating as described above gave the better yields of **14** (*R*-isomer, 11.7%) and **15** (*S*-isomer, 11.7%), the total yield being 26%. Recyclization by the method of Masamune *et al.*[11] was unsuccessful in this case. The structures of **14** and **15** were proved by the ^{1}H-NMR (250 MHz) studies. The H-14 proton resonance (multiplet, δ 3.19 for **12** and δ 3.29 for **13**), which was clearly discernable from other signals by the presence of coupling with the olefin proton at C-13, could be correlated, by the decoupling method, to the resonance of the CH$_2$ protons (H-15a,b) at δ 4—4.4 (δ 4.20 and 4.29 for **14**; δ 4.07 and 4.38 for **15**). The low-field resonances of the C-15 methylene protons as compared with those of C-23 methylene protons ($\delta \approx 3.7$) of **1** and **5** indicated that the methylenes of **14** and **15** should be bonded to a carboxyl group. The methylenes in question should originate from the 23-methylenes of **10** and **11**, because all the free hydroxyl groups (at C-3, 15,2′, and 4′) other than that at C-23 are bonded to a methine group, respectively. In the above reaction, formation of condensation products formed by linking the carboxylic acid groups with the secondary hydroxyl groups above-mentioned, or formed by intermolecular condensation should be considered, however, none of them were isolated. The fact that **14** and **15** were not the intermolecular-condensation products was verified from the physicochemical properties of **14**, **15** and other derivatives as described later. It is, therefore, concluded that ring closure occurred principally at the primary hydroxyl groups to form 16-membered lactones. It should be noted that this is the first successful macrolide recyclization of seco-acids bearing a sugar portion.

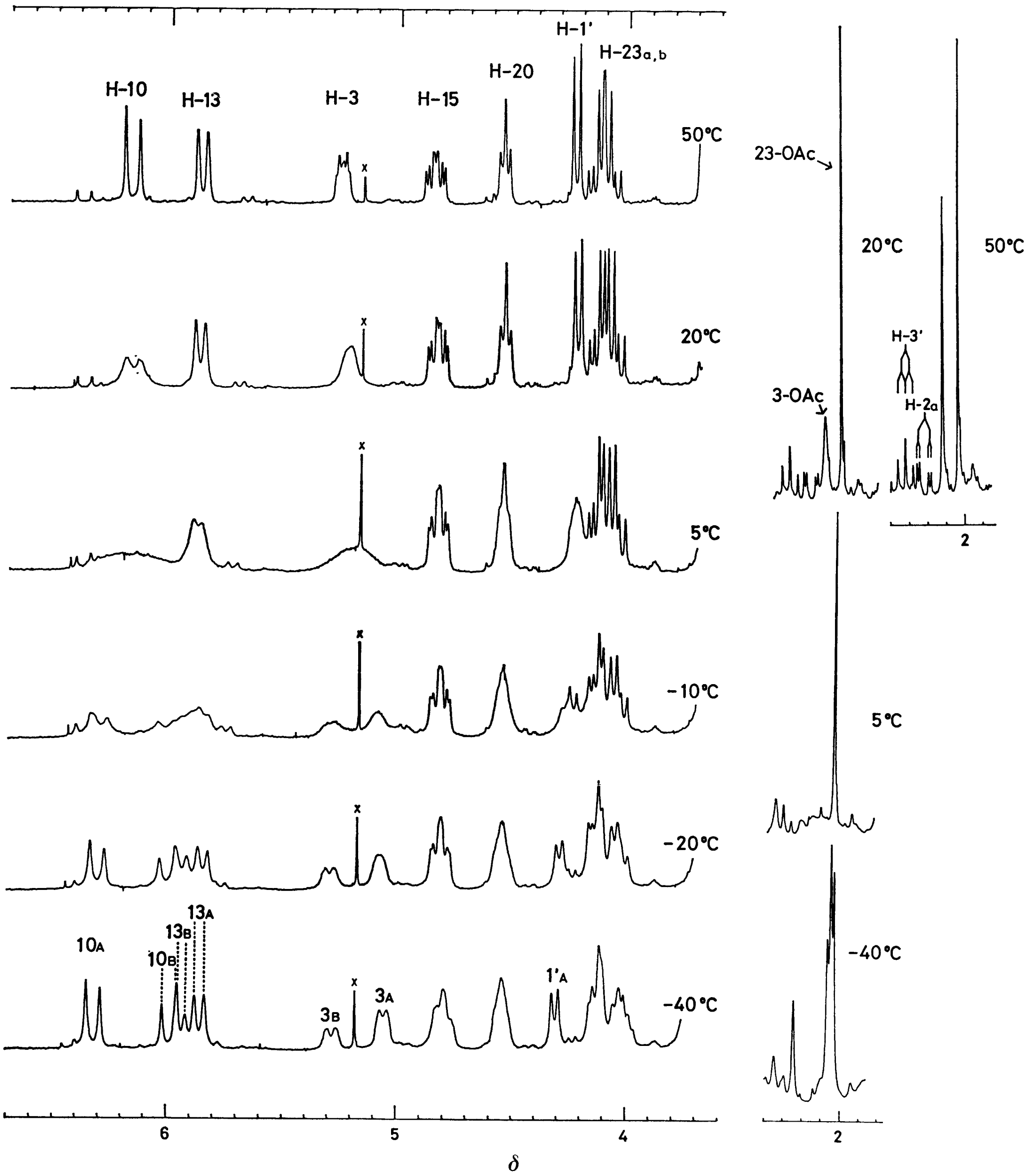


Fig. 1. Partial ^{1}H-NMR spectra of **3** in CDCl$_3$ at several temperatures. Letters of A and B indicate the signals caused by the rotamors. Impurity signals are denoted by x.

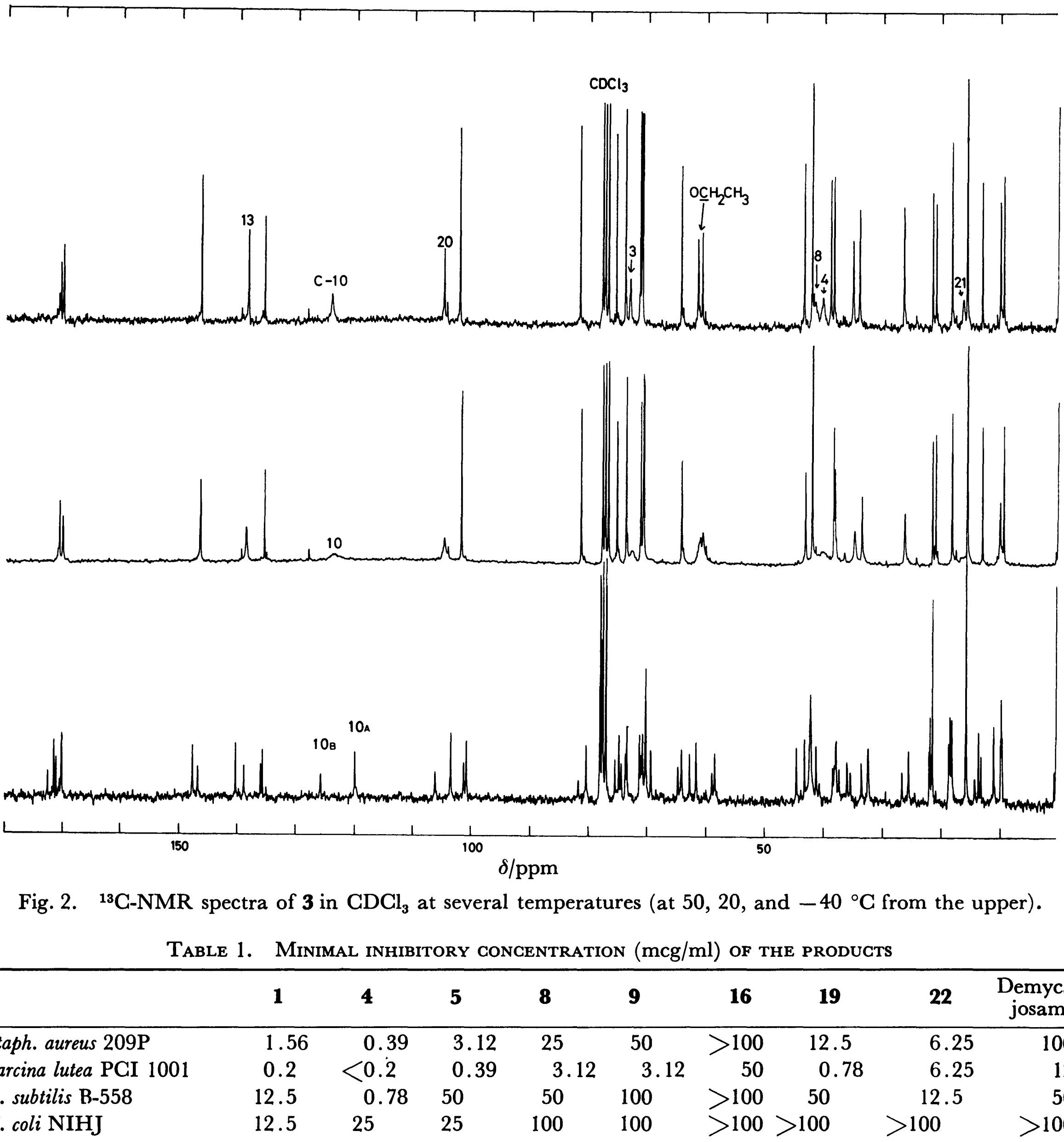


Fig. 2. ^{13}C-NMR spectra of **3** in CDCl$_3$ at several temperatures (at 50, 20, and −40 °C from the upper).

TABLE 1. MINIMAL INHIBITORY CONCENTRATION (mcg/ml) OF THE PRODUCTS

	1	**4**	**5**	**8**	**9**	**16**	**19**	**22**	Demycarosyl josamycin
Staph. aureus 209P	1.56	0.39	3.12	25	50	$>$100	12.5	6.25	100
Sarcina lutea PCI 1001	0.2	$<$0.2	0.39	3.12	3.12	50	0.78	6.25	12.5
B. subtilis B-558	12.5	0.78	50	50	100	$>$100	50	12.5	50
E. coli NIHJ	12.5	25	25	100	100	$>$100	$>$100	$>$100	$>$100
Kl. pneumoniae PCI 602	6.25	12.5	100	100	25	$>$100	100	$>$100	$>$100
Sh. dysenteriae TS 11910	0.78	0.78	3.12	3.12	12.5	50	3.12	6.25	25
Sal. enteritidis 1891	3.12	6.25	6.25	6.25	25	$>$100	6.25	12.5	50

Deacetalation of **14** gave (9*R*)-9-hydroxy compound (**16**) which is a recyclized congener of bioactive **8**. Deacetalation of **15**, however, failed to give the corresponding (9*S*)-9-hydroxy compound (**17**) owing to the difficulty of isolation of **17** from the reaction mixture.

Selective oxidation at C-9 of **14** and **15** was next performed. Oxidizing agents usually used for macrolides were, for example, active manganese dioxide[6,12] in dichloromethane, aluminium isopropoxide–cyclohexanone in toluene[13] (Oppenauer method), pyridinium chlorochromate in dichloromethane,[14] chromium trioxide in hexamethylphosphoric triamide[15] and acetic anhydride–dimethyl sulfoxide. These reagents, however, when applied to **14** and **15**, gave poor yields of the desired 9-keto derivatives possibly owing to the simultaneously occurring nonselective oxidation of the hydroxyl, dimethylamino, and olefinic groups. One successful case was met, however, when 2,3-dichloro-5,6-dicyano-*p*-benzoquinone[16] (DDQ) was used. This reagent had been reported[17] to convert allylic alcohols to the corresponding unsaturated aldehydes or ketones. Treatment of **15** (9*S* isomer) with DDQ in tetrahydrofuran gave 9-keto compound (**18**) in 47% yield. Use of benzene as solvent, which is usually used in this kind of reaction, was found to be unsuccessful. The structure of **18** was confirmed by the IR, mass, and ^{1}H-NMR spectroscopy. In the ^{1}H-NMR spectrum, no signal assignable to H-9 was observed, and H-10 olefin proton appeared as a doublet indicating again that H-9 was lacked. Similar oxidation of the 9*R* isomer (**14**), however, gave **18** in only 2.5% yield with an unresolved product mainly produced, indicating that the oxidation (hydrogen abstraction) pathway is highly dependent on the stereo-specificity. Deacetalation of **18** gave **19** which is the recyclized congener of **1**.

Several acetyl derivatives were further prepared in order to confirm the structure of **18** and to test their antibacterial activities. Acetylation of **18** gave 3,21,2′,4′-tetra-*O*-acetyl derivative (**20**), which on treatment with methanol (*vide supra*) gave 3,21-di-*O*-acetyl derivative (**21**). Deacetalation of **21** gave 3,21-di-*O*-acetyl derivative (**22**) of **19**.

Antibacterial spectra of the compounds having the aldehyde free were shown in Table 1. Comparison of the data of **19** and **1**, and **22** and **4** (*vide infra*) shows that the recyclized products have antibacterial activity lower than the mother compounds. It should be noted, however, that the recyclized compounds, except **16**, show stronger activities than that of demycarosyljosamycin which is closely related to **19**.

Several acetyl derivatives of **1** were also prepared as reference compounds for biological test. Mycaminosyltylonolide diethyl acetal (**2**) was treated with acetic anhydride in pyridine to give 3,23,2′,4′-tetra-*O*-acetyl derivative, which, on treatment with methanol,[18] gave 3,23-di-*O*-acetyl derivative (**3**), showing that the 2′- and 4′-*O*-acetyl groups were selectively removed. Acidic hydrolysis of **3** in aqueous acetonitrile gave deacetalated 3,23-di-*O*-acetyl derivative (**4**). Acidic hydrolysis of **3** in slightly stronger conditions gave 3-*O*-acetyl derivative (**5**).

Here we describe the conformational feature of **3** detected by ^{1}H-NMR spectroscopy by varying the temperature (see Fig. 1). At 50 °C in chloroform-*d*, all proton signals appeared as sharp lines, and at lower temperatures, they gradually broadened. Among them, the singlet of 3-*O*-acetyl (δ 2.12) showed rapid broadening; the ratio of the height of the acetyl signal for that of 23-*O*-acetyl (δ 2.03) changed as follows: 0.66 (at 50 °C), 0.42 (44 °C), 0.34 (34 °C), 0.14 (20 °C). At 0 °C the 3-*O*-acetyl signal melted away not to be detectable. At −10 °C, the acetyl peak at δ 2.08 (*ca.* 4.5H in strength) still kept sharp line, but a broadened peak (*ca.* 1.5H) newly appeared at δ 2.26. At −20 °C the latter peak sharpened a little, and at −40 °C all peaks assignable to acetyls appeared as four sharp singlets at δ 2.09 (major), 2.10 (major), 2.12 (minor), and 2.29 (minor, *ca.* 1.2H). The H-3 signals (δ 5.23) also behaved similarly. The sharp multiplet (apparently double triplets of $J_{2b,3}$ 8.5 Hz, $J_{2a,3} \simeq J_{3,4} \simeq 4$ Hz) at 50 °C gradually broadened at lower temperatures, and at 20 °C it became a broadened singlet. At 0 °C it melted away (δ 5.0—5.4). At that temperature, the following signals still kept sharp lines or slightly broadened: methyls of 17, 21, 22, 6′; 23-*O*-acetyl, dimethylamino, $-OCH_2C\underline{H}_3$; methylenes of 16, 23; methines of 15 and 3′. At −10 °C, line-resharpening of all signals began, and H-3 signals were separated into two broadened singlets at δ 5.13 and 5.33 (*ca.* 1 : 1 in strength), which, at lower temperatures, gradually sharpened to become two doublets (J 7.5 and 9 Hz) in the strength of *ca.* 1.2 : 1 (−20 °C), 1.5 : 1 (−40 °C), and 2 : 1 (−55 °C) without substantial change in the shift-values. Other signals separated into two parts were those of H-10 (eminent below −10 °C), -11 (below −20 °C), -13 (below −20 °C), 18-methyl (below −20 °C), and H-1′ (−20 °C). Other signals which showed great broadening at low temperatures were the peaks of H-4 (broadening began at 34 °C, and at 0 °C, melted away), H-10 (broadening began at 20 °C), H-2a and -2b (broadening began both at 10 °C and melted away at 0 °C), 18-methyl (broadening began at 10 °C), and possibly H-7.

Above data can be summarized as follows: 1) line-broadening occurred not at a time, the signals of H-3, 3-*O*-acetyl, H-4, and H-10 being first broadened, then those of 18-methyl, H-2a, and -2b followed, 2) the signals at C-15, 16, 17, 21, 23, 3′, 6′, of $(OCH_2C\underline{H}_3)_2$, 23-*O*-acetyl, and $N(CH_3)_2$ resisted to the broadening, and 3) at low temperatures, the signals of H-3, 10, 11, 13, and 3-*O*-acetyl separated into two parts with varying the strength-ratio with the temperatures.

These data disclosed the conformational feature of **3** dissolved in chloroform-*d*. First, it is concluded, from 3), that **3** will have two rotational isomers on the NMR time scale, and at 55 °C, for example, they show faster interconversion to give a clear spectrum. The situation is typically shown by the fact that the J values relating

TABLE 2. THE ^{13}C-NMR CHEMICAL SHIFTS OF **1**, **3**, AND **4** IN $CDCl_3$ (in ppm downfield from TMS)

	1 (at 20 °C)	**3** (at 50 °C)	**4** (at 20 °C)
C-1	173.85	170.08	170.20
C-2	39.54	38.09	37.78
C-3	67.8(v. br)	72.79(small)	68.60(small)
C-4	47.14	39.94(v. br)	43.33(small)
C-5	81.15(br)	81.47	80.66
C-6	31.96(br)	38.61	33.01
C-7	32.75(br)	33.77[a]	33.01
C-8	40.42(br)	41.14(v. br)	40.17
C-9	203.61	204.40	203.56
C-10	118.72(br)	123.91(br)	120.35(small)
C-11	148.14	146.36	147.77
C-12	135.65	135.41	135.63
C-13	142.10	138.31	139.64
C-14	44.61	43.05	42.99
C-15	75.10	75.20	74.64
C-16	25.52	26.24	25.80
C-17	8.98	9.24	9.22
C-18	9.68	9.80	10.25
C-19	43.75	34.82[a]	44.21
C-20	203.02	104.62	202.36
C-21	17.35	16.03(br)	17.19
C-22	13.06	12.86	13.05
C-23	62.73	64.20	63.98
$OCO\underline{C}H_3$		20.67, 21.31	20.80, 21.27
$O\underline{C}OCH_3$		170.53, 170.78	170.80, 170.94
$OCH_2\underline{C}H_3$		15.43	
$O\underline{C}H_2CH_3$		60.64, 61.39	
C-1′	104.03	101.90	104.20
C-2′	70.90	70.70	71.15
C-3′	70.16	70.87	70.22
C-4′	70.90	71.16	70.88
C-5′	73.32	73.67	73.58
C-6′	17.81	18.01	17.84
NMe_2	41.68	41.77	41.75

a) May be interconvertible.

to H-2a, 2b, 3, and 4 is changed at the temperatures above and below 5 °C. The coalescence point is estimated to be near 5 °C, the (high) value being unexpected for the macrolide derivative. From 2) and 3) it is suggested that the origin of the energy barrier for the interconversion between the rotamers might be principally created by the steric crowd of the substituents between C-3—C-8 including 3-*O*-acetyl. The diene portion will come near to the above portion (C-3—C-8) and be influenced by the above rotational changes. The positions remote from the above portion (C-3—C-8) will not much be influenced, keeping always similar spectral environments.

Above conclusions were basically confirmed by the ^{13}C-NMR spectroscopy. The spectra of **3** measured at various temperatures are shown in Fig. 2. Signal assignments (see Table 2) were made on the basis of the comparison of the spectrum with that of tylosin[19] and also by off-resonance method as well as by selective proton decouplings (see Experimental). In the spectrum, the signals of ^{13}C-3, 4, 8, and 10 appeared, even at 50 °C, as low and broadened peaks. At 20 °C, the signals of ^{13}C-18 (slight), 16 (slight), 19, $O\underline{C}H_2CH_3$, 20, 13, and 9 were additionally broadened. At −40 °C, most of the carbon signals split into doublets of unequal intensities. These results indicate again that **3** has two major rotational isomers.

Experimental

General. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Thin-layer chromatography (TLC) was carried out on Wakogel B5 silica gel with detection by spraying with sulfuric acid, followed by slight heating. Column chromatography was performed on Kiesel gel 60, 230—400 mesh (E. Merck). UV spectra were recorded with a Hitachi 200-10 spectrometer, and IR spectra, with a Hitachi 255 grating spectrometer. ^{1}H-NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer, or at 250 MHz in the FT mode using a Bruker WM 250 spectrometer at 20 °C unless otherwise stated. ^{13}C-NMR spectra were recorded in the FT mode with a Bruker WM 250 spectrometer operating at 62.9 MHz.

*^{1}H-NMR(in $CDCl_3$ at 250 MHz) of **1**:* δ 0.95 [3H t, Me(17)], 1.02 [3H d, Me(18)], 1.22 [3H d, Me(21)], 1.27 [3H d, Me(6′)], 1.83 [3H very narrow m; apparently s, Me(22)], 1.96 (1H d, $J_{2a,2b}$ 15 Hz, H-2a), 2.36 (1H t, J 10 Hz, H-3′), 2.50 (6H s, NMe_2), 2.53 (1H dd, $J_{2b,3}$ 10 Hz, H-2b), 2.6 (1H m, H-8), 2.9 (H-14), 3.06 (1H t, $J \simeq 9.3$ Hz, H-4′), 3.26 (1H m, H-5′), 3.47 (1H dd, $J_{1',2'}$ 7.5 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 3.73 (1H, H-5), 3.75 (2H d, J 5 Hz, H-23a,b), 3.85 (1H br d, $J \simeq 10$ Hz, H-3), 4.25 (1H d, H-1′), 4.95 (1H dt, $J_{14,15}=J_{15,16a} \simeq 9$ Hz, $J_{15,16b}$ 2.5 Hz, H-15), 5.92 (1H slightly br, d, $J_{13,14}$ 10 Hz, H-13), 6.30 (1H d, J 15 Hz, H-10), 7.34 (1H d, H-11), 9.70 (1H s, CHO).

Irradiation at δ 1.65 (H-4, 16a, and possibly one of H-7a, 7b) caused the triplet of H-17 and the doublet of H-18 to collapse to a doublet and a singlet, respectively; at the same time, signals both at δ 1.88 (H-16b) and of H-5 to change. Irradiation at δ 2.6 (H-2b, -8) collapsed the doublets of H-2a, -3, and -21 to singlets respectively; the signals near δ 1.5 (H-7a,7b) also changed. Irradiation at δ 2.9 (H-14 and -19?) caused the doublet of H-23, the doublet of H-13, and the double triplets of H-15 to collapse to a singlet, a singlet, and a doublet, respectively.

^{13}C-NMR (in $CDCl_3$ at 20 °C). (s, d, and t cited in the parenthesis are the splitting pattern in the off-resonance). Carbon- 17, 18, 22, 21, 6′, 16(t), 6(d), 7(t), 2(t), 8(d), NMe_2, 19(t), 5(d), 1′(d), 10(d), 12(s), 13(d), 11(d), 1(s), 20(d), 9(s) (see Table 2.) were assigned on the bassi of the comparison of the shift-values with those of tylosin[19] as well as by off-resonance method. Carbon-23 can be assigned by the off-resonance method (appeared as a triplet) as well as by the appearance at an up-field shift (62.73 ppm) in comparison to the shift (68.2 ppm) of C-23 of tylosin, the shift being in accord with the removal of the glycoside linkage from tylosin. On H-3′ proton irradiation, C-3′ appeared as a singlet; the splitting spaces of the signals (each doublet) of C-4′, 2′ and 5′, 3 and 15 became wider in this order, which were proportional to the order of the $\Delta\delta$ value of the protons (H-4′, 2′, 5′, 3, and 15) from the δ value of H-3′. This observation assisted the carbon assignments in question. Distinction between C-4 and -14 was performed by the H-14 proton irradiation.

Mycaminosyltylonolide Diethyl Acetal (2). A solution of **1** (825 mg) in dry ethanol (8.3 ml) containing anhydrous *p*-toluenesulfonic acid (360 mg) was kept at room temperature for 20 min. After addition of triethylamine (0.3 ml), the solution was concentrated and the residue was dissolved in chloroform. The solution was washed with saturated aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated to give a solid. Recrystallization from acetone solution by adding hexane gave needles, 852 mg (92%), mp 193—195 °C, $[\alpha]_D^{25}$ +14° (*c* 1, chloroform); UV λ_{max} (MeOH) 283 nm (log ε 4.40).

Found: C, 62.29; H, 8.95; N, 2.13%. Calcd for $C_{35}H_{61}NO_{11}$: C, 62.57; H, 9.15; N, 2.08%.

3,23-Di-O-acetylmycaminosyltylonolide Diethyl Acetal (3). Treatment of **2** (992 mg) in pyridine (10 ml) with acetic anhydride (2.5 ml) at 50 °C for 2 d gave 3,23,2′,4′-tetra-*O*-acetyl derivative, which gave, on TLC with benzene–acetone (5 : 1), a spot of R_f 0.35. The crude derivative was dissolved in methanol and the solution was kept at 50 °C overnight to give an amorphous solid of **3**, 1.05 g (94%); R_f 0.5 on TLC with chloroform–methanol (7 : 1) (the tetraacetyl intermediate: R_f 0.95), $[\alpha]_D^{25}$ +3° (*c* 1, chloroform).

Found: C, 62.15; H, 8.59; N, 1.88%. Calcd for $C_{39}H_{65}NO_{13}$: C, 61.97; H, 8.64; N, 1.85%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz) (at 50 °C): δ 0.93 [3H t, Me(17)], 1.03 [3H d, Me(18)], 1.15 [3H d, Me(21)], 1.15—1.24 [6H, $(OCH_2C\underline{H}_3)_2$], 1.31 [3H d, Me(6′)], $\simeq$1.35—1.5 (H-19a), $\simeq$1.37—1.52 (H-7), 1.61 (1H m, H-16a), $\simeq$1.75 (H-4), $\approx$1.8 (H-16b), 1.81 [3H s, Me(22)], $\approx$2.0 (H-6, 19b), 2.03 (3H, s, 23-*O*-Ac), 2.12 (3H s, 3-*O*-Ac), 2.22 (1H dd, $J_{2a,3}$ 3.3 Hz, $J_{2a,2b}$ 15 Hz, H-2a), 2.32 (1H t, H-3′), 2.51 (6H s, NMe_2), 2.57 (1H dd, $J_{2b,3}$ 8.5 Hz, H-2b), 2.95—3.07 (3H, H-8, 14, 4′), 3.29 (1H m, H-5′), 3.44—3.55 [$(OC\underline{H}_2CH_3)_2$], 3.59 (1H dd, H-2′) $\approx$3.67 (H-5); 4.08 (1H dd with the lower-field half being intense, $J_{14,23a}$ 6.5 Hz, $J_{23a,23b}$ 11.5 Hz, H-23a), 4.14 (1H dd with the higher-field half being intense, $J_{14,23b}$ 5 Hz, H-23b), 4.23 (1H d, $J_{1',2'}$ 7.5 Hz, H-1′), 4.54 (1H apparent t, H-20), 4.84 (1H ddd, H-15), 5.25 (1H m, H-3), 5.86 (1H d, $J_{13,14}$ 10 Hz, H-13), 6.17 (1H d, J 15.5 Hz, H-10), 7.25 (1H d, H-11).

Irradiation of 17-methyl collapsed the multiplet of H-16a to double doublets ($J_{15,16a}$ 9.5, $J_{16a,16b}$ 15 Hz), and the signals between δ 1.77—1.86 (H-16b) changed. Irradiation at δ 1.82 (H-16b and 4) caused the triplet of H-17, the multiplet of H-15, the multiplet of H-3 and the doublet of CH_3 (18) to collapse to a doublet, double doublets ($J_{14,15}$ and 9.5 Hz), double doublets, and a singlet, respectively; the signals of

H-16a also changed although they were biased by the near-by irradiation. Irradiation at δ 3.0 (H-8, 14, and 4′) collapsed the doublet of Me(21) to a singlet, the multiplet of H-23a, -23b to an AB quartet, the doublet of H-13 to a singlet, and the multiplet of H-15 to double doublets; the signals of H-5′ and -3′ also changed to the form as expected. Irradiation of H-3 caused the signals of H-2a, -2b to collapse to an AB quartet and the signals near δ 1.75 (H-4) to change. Irradiation at δ 3.67 (H-5) caused the signals of H-4 to change. Irradiation at δ 1.95 (H-6, 19b) caused the signals between δ 1.35—1.53 (H-7, 19b) to simplify, and the triplet of H-20 to a doublet. To confirm the signal assignments, the positions of H-15, 20, and 1′ were also irradiated.

At −20 °C: 18-Methyl separated into two incomplete doublets at δ 1.01 and 1.10. H-1′ separated into two, the bigger half being appeared at δ 4.33 (J 7.5 Hz). H-13 gave two doublets (each $J\simeq10$ Hz) at δ 5.88 and 5.97. H-10 gave two doublets (each $J\simeq15$ Hz) at δ 6.03 and 6.34. H-11 gave apparent triplet at δ 7.34.

At −40 °C: 18-Methyl gave two doublets at δ 1.01 (J 6.3 Hz) and 1.12 (J 5 Hz). The bigger signals of H-1′ appeared at δ 4.35 ($\simeq$0.6H d, J 7.5 Hz). H-3 gave two slightly broaened doublets at δ 5.09 ($\simeq$0.6H, $J\simeq7.5$ Hz) and 5.33 ($\simeq$0.4H, $J\simeq10$ Hz). H-13 gave two sharp doublets (J 10 Hz) at δ 5.90 ($\simeq$0.6H) and 5.98 ($\simeq$0.4H). H-10 gave two sharp doublets (J 15.5 Hz) at δ 6.03 ($\simeq$0.4H) and 6.37 ($\simeq$0.6H).

^{13}C-NMR (in $CDCl_3$ at 50 °C). Carbon-17, 18, 22, $(\underline{C}H_3CH_2O-)_2$(q, each), 21, 6′, $(\underline{C}H_3CO_2-)_2$, 16(t), NMe_2(q), 14(d), $(CH_3\underline{C}H_2O-)_2$ (t, each), 23, 5, 1′, 20(d), 10, 12, 13, 11, 1, $(CH_3\underline{C}O_2-)_2$, 9(s) (see Table 2) were assigned on the basis of the comparison of the shift-values with those of **4** as well as by the off-resonance. The carbons ranging between 30—45 ppm (C-2, 4, 6, 7, 8, 14, and 19) could be classified into two groups of methylene (C-2, 7, 19) and methine (C-4, 6, 8, 14) by the method of off-resonance. By the proton irradiation at δ 1.3, C-2(d) could be distinguished from C-7(s) and 19(s). By the H-14(16 and 22) proton irradiation, C-4(s) and C-6 (narrow d) could be distinguished from C-8 and 14. The above assignments were also confirmed by the proton irradiations of H-6 and of 14(8 and 4′). By the latter irradiation, also, assignments of carbons (between 70—76 ppm) of 4′(s), 5′(very narrow d), 2′(narrow d), 3′(d), 15 and 3(wide d, each) were made. The above assignments were substantiated by the observation that the $^1J_{C-H}$ values in question came roughly in the range of similar magnitudes (by gated decoupling method). Carbon-3 was decided by the H-3 proton irradiation.

*3,23-Di-*O*-acetylmycaminosyltylonolide (**4**).* A solution of **3** (100 mg) in a mixture of acetonitrile (2 ml) and 0.1 M aqueous hydrochloric acid (2 ml) was kept at room temperature for 1 h. After addition of sodium hydrogencarbonate (34 mg) and water (4 ml), the reaction mixture was extracted with chloroform. The crude product obtained was purified by chromatography on a short column of silica gel with chloroform–methanol (7 : 1) to give an amorphous solid of **4**, 86 mg (96%), $[\alpha]_D^{25}$ +19° (c 0.7, chloroform).

Found: C, 61.83; H, 8.35; N, 2.32%. Calcd for $C_{35}H_{55}NO_{12}$: C, 61.66; H, 8.13; N, 2.05%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 0.94 [3H t, Me(17)], 1.09 [3H d, Me(18)], 1.23 [3H d, Me(21)], 1.27 [3H d, Me(6′)], 1.83 [3H s, Me(22)], 1.78—1.92 (H-4, 16b), 2.05 (3H s, 23-*O*-Ac), 2.15 (3H s, 3-*O*-Ac), 2.35 (1H t, H-3′), 2.50 (6H s, NMe_2), 2.64 (1H dd, H-2), 2.98—3.1 (H-14), 3.07 (1H t, H-4′), 3.27 (1H dd, H-5′), 3.48 (1H dd, H-2′), 3.57 (1H d, H-5), 4.09 (1H dd, H-23a), 4.16 (1H dd, H-23b), 4.21 (1H d, H-1′), 4.82 (1H ddd, H-15), 5.18 (1H unresolved d, $J\simeq10$ Hz, H-3), 5.90 (1H d, H-13), 6.30 (1H d, H-10), 7.43 (1H d, H-11), 9.65 (1H s, CHO). Above assignments were confirmed by the decoupling method.

^{13}C-NMR (in $CDCl_3$ at 20 °C). Carbon-17, 18, 22, 21, 6′, $(\underline{C}H_3CO_2-)_2$ (q, each), 16(t), 6, 7, 2(t), 8(d), NMe_2, 19(t), 23(t), 3(d), 5′, 15, 5, 1′, 10, 12, 13, 11, 1, $(CH_3\underline{C}O_2-)_2$ (s, each), 20(d), 9(s) (see Table 2.) were assigned on the basis of the comparison of the shift-values with those of **1** as well as by the off-resonance method. Carbon-15 was confirmed by the H-15 proton irradiation. Distinction between C-4 and -14 was performed by the H-14 proton irradiation. Distinction between C-2′, 3′, and 4′ was performed by the H-4′ proton irradiation, whereupon C-4′ appeared as a singlet, and C-2′ and 3′ as a doublet, respectively, but the peak space of the latter doublet was wider than that of the former one (see the similar statement of ^{13}C-NMR of **3**). Since $^1J_{C2'-H2'}$ and $^1J_{C3'-H3'}$ were observed to be almost equal (by the gated decoupling technique), assignments of C-2′ and 3′ by the above space-method will be resonable.

*3-*O*-Acetylmycaminosyltylonolide (**5**).* An aqueous solution (3.5 ml) of **3** (69 mg) and *p*-toluenesulfonic acid monohydrate (26 mg) was kept at 65 °C for 20 h. After addition of sodium hydrogencarbonate (20 mg), the reaction mixture was extracted with chloroform. The crude product obtained showed, on TLC with chloroform–methanol-28% aqueous ammonia (10 : 1 : 0.1), three spots of R_f 0.4 (slight **3**), 0.3 (major, **5**), and 0.22 (slight, 23-*O*-acetyl derivative?). Silica-gel column chromatography with chloroform–methanol–28% aqueous ammonia (20 : 1 : 0.1) gave an amorphous solid of **5**, 30 mg (51%), $[\alpha]_D^{18}$ +15° (c 1, chloroform).

Found: C, 62.17; H, 8.46; N, 2.18%. Calcd for $C_{33}H_{53}NO_{11}$: C, 61.95; H, 8.35; N, 2.19%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 1.85 [3H s, Me(22)], 2.15 (3H s, 3-*O*-Ac), 2.50 (6H s, NMe_2), 3.66 (1H dd, $J_{14,23a}$ 7 Hz, $J_{23a,23b}$ 11.5 Hz, H-23a), 3.73 (1H dd, $J_{14,23b}$ 5 Hz, H-23b), 4.21 (1H d, H-1′), 4.80 (1H m, H-15), 5.20 (1H unresolved d, $J\simeq11$ Hz, H-3), 5.93 (1H d, H-13), 6.29 (1H d, H-10), 7.41 (1H d, H-11), 9.65 (1H s, CHO).

Irradiation at δ 2.93 (H-14) collapsed the signals of H-23a, 23b to an AB quartet, and the doublet of H-13 to a singlet.

*(9*R*)-9-Deoxo-9-hydroxy- (**6**) and (9*S*)-9-Deoxo-9-hydroxymycaminosyltylonolide Diethyl Acetal (**7**).* To an ice-cold solution of **2** (567 mg) in methanol (5.7 ml) was added sodium borohydride (96 mg) and the solution was kept in the cold for 3 h. On TLC with chloroform–methanol–28% aqueous ammonia (12 : 1 : 0.1), the reaction mixture showed spots of R_f 0.37 (**6**), 0.33 (**7**) (the color-intense was equal), and 0.3 (very slight), the spot of **2** (R_f 0.4) being disappeared. After concentration, the residue was extracted with chloroform and the solution was washed with water, dried, and concentrated. Column chromatography of the residue with chloroform–methanol–28% aqueous ammonia (15 : 1 : 0.1) gave amorphous solids of **6** (73 mg, 13%), **7** (86 mg, 15%) and the mixture of the two (383 mg, 67%).

6: $[\alpha]_D$ +38° (c 1, chloroform); UV λ_{max} (MeOH) 237 nm (log ε 4.57).

Found: C, 62.11; H, 9.20; N, 2.08%. Calcd for $C_{35}H_{63}NO_{11}$: C, 62.38; H, 9.42; N, 2.08%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 1.78 [3H s, Me(22)], 2.51 (6H s, NMe_2), 4.28 (1H broadened d; at 50 °C: sharp dd, $J_{8,9}$ 2.5 Hz, $J_{9,10}$ 8.0 Hz, H-9), 4.34 (1H d, H-1′), 4.65 (1H unresolved m; at 50°C, sharp dd, J 4 and 6.5 Hz, H-20), 4.89 (1H dt, H-15), 5.24 (1H d, J 10 Hz, H-13), 5.76 (1H dd, $J_{10,11}$ 15.5 Hz, H-10), 6.15 [1H br ($\simeq$6.0—6.3) s; at 50 °C, sharp d, J 15.5 Hz, H-11].

Irradiation (at 50 °C) of H-10 collapsed the signals of H-9 and H-11 to a small doublet, and a singlet, respectively. Irradiation (at 50 °C) at δ 1.9 (H-8) collapsed the double doublets of H-9 to a doublet (J 8 Hz).

7: $[\alpha]_D^{25}$ +37° (c 1, chloroform); UV λ_{max} (MeOH) 237 nm (log ε 4.41).

Found: C, 62.56; H, 9.27; N, 2.16%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 1.78 (3H s, Me(22)), 2.51 (6H s, NMe_2), 4.32 (1H d, H-1′), 4.61 (H-20), 4.90 (H-15), 5.29 (1H d, J 10 Hz, H-13), 5.95 (1H br ($\simeq$5.85—6.1) s; at 50 °C: sharp dd, $J_{9,10}$ 4.5 Hz, $J_{10,11}$ 15.5 Hz, H-10), 6.47 (1H d, H-11). Signals of H-1′, -20, -15, -13, and -11 slightly sharpened at 50 °C.

Irradiation (at 50 °C) of H-10 collapsed the doublet of H-11 to a singlet. Irradiation (at 50 °C) at δ 3.92 (H-9) collapsed the double doublets of H-10 to a doublet.

*9-Deoxo-9(*R*)-hydroxy- (**8**) and 9-Deoxo-9(*S*)-hydroxy-mycaminosyltylonolide (**9**).* To a solution of **6** or **8** (40 mg, 0.06 mmol) in acetonitrile (1.2 ml) was added 0.1 M aqueous hydrochloric acid (1.2 ml) and the solution was kept at room temperature for 1 h. After addition of aqueous saturated sodium hydrogencarbonate (1.2 ml), the mixture was extracted with chloroform. The organic solution was washed thoroughly with water, dried over sodium sulfate, and concentrated. The residue was chromatographed on a short column of silica gel with chloroform–methanol–28% aqueous ammonia (10 : 1 : 0.1) to remove the slightly remained starting material to give an amorphous solid of **8** (30.5 mg, 86%) or **9** (31.5 mg, 88%).

8: $[\alpha]_D^{25}$ +44° (c 0.5, chloroform). ^{1}H-NMR ($CDCl_3$): δ 4.23 (1H dd, $J_{8,9}$ $\simeq$2 Hz, $J_{9,10}$ 8 Hz, H-9), 4.94 (1H dt, H-15), 5.33 (1H d, $J_{13,14}$ 10 Hz, H-13), 5.78 (1H dd, H-10), 6.25 (1H d, $J_{10,11}$ 16 Hz, H-11), 9.86 (1H s, H-20).

Found: C, 62.28; H, 9.01; N, 2.26%. Calcd for $C_{31}H_{53}NO_{10}$: C, 62.08; H, 8.91; N, 2.34%.

9: $[\alpha]_D^{25}$ +34° (c 0.5, chloroform). ^{1}H-NMR ($CDCl_3$): δ 4.95 (1H dt, H-15), 5.37 (1H d, H-13), 5.96 (1H dd, $J_{9,10}$ 4 Hz, $J_{10,11}$ 16 Hz, H-10), 6.57 (1H d, H-11), 9.80 (1H s, H-20).

Found: C, 61.99; H, 8.86; N, 2.24%. Calcd for $C_{31}H_{53}NO_{10}$: C, 62.08; H, 8.91; N, 2.34%.

*Basic Hydrolysis of the Mixture of **6** and **7** (to Give **10** and **11**).* To a solution of a mixture of **6** and **7** (4.95 g) in methanol (92 ml) was added 0.4 M aqueous sodium hydroxide (92 ml) and the mixture was heated at 50 °C for 5 h. Addition of 1 M aqueous hydrochloric acid (37 ml) followed by concentration to $\approx$80 ml gave precipitates of sodium chloride, which was filtered and washed with methanol. The filtrate and the washings combined were concentrated, filtered from sodium chloride further precipitated, and the concentrate was chromatographed on a column of Sephadex LH-20 (pretreated with methanol) with methanol to give a mixture of sodium chloride-free products (**10**, **11**), 4.56 g (91%).

*Esterification of **10** and **11** (to Give **12** and **13**).* To a solution of a mixture of **10** and **11** (142 mg) in dry tetrahydrofuran (1.4 ml) was added bis(4-*t*-butyl-1-isopropyl-2-imidazolyl) disulfide (124 mg) and triphenylphosphine (82 mg) under argon atmosphere and the mixture was kept at room temperature for 5 min. Methanol (14 ml) was added and the solution was kept overnight at room temperature. The products obtained were chromatographed with chloroform–methanol–28% aqueous ammonia (9 : 1 : 0.1) to give a mixture of **12** and **13**, 78.5 mg (53%). The mixture was further chromatographed (8 g of silica gel) in a similar manner to give **11** (R_f 0.32 on TLC with chloroform–methanol–28% aqueous ammonia = 10 : 1 : 0.1), 10.9 mg, and **12** (R_f 0.28), 15.4 mg, and the mixture of the two.

11: $[\alpha]_D^{21}$ −4° (c 0.5, chloroform); m/e 705 (M^+), 659. Found: C, 61.08; H, 9.41; N, 2.00%. Calcd for $C_{36}H_{67}NO_{12}$: C, 61.25; H, 9.57; N, 1.98%.

^{1}H-NMR ($CDCl_3$): δ 3.72 (3H s, CO_2Me).

12: $[\alpha]_D^{21}$ −10° (c 0.5, chloroform): m/e 659. Found: C, 61.16; H, 9.34; N, 2.13%.

^{1}H-NMR ($CDCl_3$): δ 3.72 (3H s, CO_2Me).

*Cyclization of **10** and **11** (to Give **14** and **15**).* *Method A*: To a solution of a mixture of **10** and **11** (30.6 mg, 0.044 mmol) in dry tetrahydrofuran (0.6 ml) was added, under argon atmosphere, 2,2′-dipyridyl disulfide (14.6 mg, 0.066 mmol) and triphenylphosphine (17.4 mg, 0.063 mmol) and the mixture was kept at room temperature for 30 min. Concentration gave a residue, which was dissolved in dry toluene (16 ml) and the solution was heated at 80 °C for 24 h. The reaction mixture showed, on TLC, two spots of R_f 0.25 (**14**) and 0.28 (**15**) (with 10 : 1 : 0.1 chloroform–methanol–28% aqueous ammonia) accompanied by tailing zones of R_f 0—0.18 and 0.34—0.45. The solution was concentrated and the residue was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate, and concentrated. The residue was chromatographed on a silica-gel column (6 g) with chloroform–methanol–28% aqueous ammonia (12 : 1 : 0.1) to give a mixture of **14** and **15**, 5.1 mg (17%).

Method B: To a solution of a mixture of **10** and **11** (4.56 g, 6.59 mmol) in dry tetrahydrofuran (90 ml) was added bis-(4-*t*-butyl-1-isopropyl-2-imidazolyl) disulfide (3.91 g, 9.88 mmol) and triphenylphosphine (2.60 g, 9.88 mmol) under arogon atmosphere and the mixture was kept at room temperature for 5 min. The reaction mixture showed, on TLC with 6 : 1 chloroform–methanol, spots of R_f 0.3 (active ester) and 0 (hydrolyzed products of the active esters?). Concentration gave a residue, which was dissolved in toluene (1140 ml) and the solution was heated at 80 °C for 24 h. The TLC pattern of the reaction mixture was substantially the same with that obtained by "Method A". Similar work-up as described in "Method A" gave an amorphous powder, which was chromatographed on a silica-gel column (Wakogel C-200, 450 g) with chloroform–methanol–28% ammonia (10 : 1 : 0.1) to give **14** (0.52 g, 11.7%), **15** (0.52 g, 11.7%), and a mixture of **14** and **15** (0.10 g, 2.3%).

14: $[\alpha]_D^{25}$ −6° (c 0.5, chloroform): UV λ_{max} (MeOH) 236 nm (log ε 4.26); IR(KBr): 1640, 1730 cm^{-1}.

Found: C, 62.44; H, 9.19; N, 2.18%. Calcd for $C_{35}H_{63}NO_{11}$: C, 62.38; H, 9.42; N, 2.08%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): 0.96 [3H d, Me(19 or 16)], 0.98 [3H t, Me(23)], 1.13 [3H d, Me(16 or 19)], 1.19 and 1.21 [each 3H t, $OCH_2C\underline{H}_3)_2$], 1.32 [3H d, Me(6′)], 1.78 [3H d, $J\simeq1$ Hz, Me(20)], 2.26 (1H dd, $J_{2a,2b}$ 14 Hz, $J_{2a,3}$ 4 Hz, H-2a), 2.37 (1H dd, $J_{2b,3}$ 7.5 Hz, H-2b), 2.38 (1H t, H-3′), 2.50 (6H s, NMe_2), 3.02 (1H t, H-4′), 3.19 (1H m, H-14), 3.35 (1H dd, H-5′), 4.2 (1H t, $J_{14,15a}=J_{15a,15b}$ 10.5 Hz, H-15a), 4.29 (1H dd, $J_{14,15b}$ 6 Hz, H-15_b), 4.32 (1H d, J 7.5 Hz, H-1′), 4.62 (1H unresolved t, H-18), 5.38 (1H slightly br d, $J_{13,14}$ 10 Hz, H-13), 5.55 (1H dd, $J_{9,10}$ 9 Hz, $J_{10,11}$ 15 Hz, H-10), 6.18 (1H sharp d, H-11).

Irradiation of H-14 caused the signals of H-15a, -15b to collapse to an AB quartet, the doublet of H-13 to a singlet. Irradiation at δ 3.58 (includes H-9) collapsed the signals of H-10 to a doublet. Irradiation at δ 1.45 [includes H-22 and -4(or 8)] collapsed the triplet of Me(23) and the doublet at δ 1.13 to singlets. Irradiation at δ 1.67 (H-8 or 4) collapsed the doublet at δ 0.96 to a singlet. Irradiation of H-5′ collapsed the doublet of Me(6′) and the triplet of H-4′ to a singlet and a doublet, respectively.

15: $[\alpha]_D^{25}$ −4° (c 0.5, chloroform); UV λ_{max} (MeOH)

236 nm (log ε 4.23); IR(KBr): 1640, 1730 cm^{-1}.

Found: C, 62.07; H, 9.20; N, 2.07%. Calcd for $C_{35}H_{63}NO_{11}$: C, 62.38; H, 9.42; H, 2.08%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 0.94 [3H d, Me(19 or 16)], 0.98 [3H t, Me(23)], 1.18 [3H d, Me(16 or 19)], 1.18 and 1.19 [each 3H t, $(OCH_2C\underline{H}_3)_2$], 1.31 [3H d, Me(6′)], 1.76 [3H d, $J\simeq 1$ Hz, Me(20)], 2.21 (1H dd, $J_{2a,2b}$ 14 Hz, $J_{2a,3}$ 4 Hz, H-2a), 2.34 (1H dd, $J_{2b,3}$ 9.5 Hz, H-2b), 2.38 (1H t, H-3′), 2.50 (6H s, NMe_2), 3.01 (1H t, H-4′), 3.29 (1H m, H-14), 3.34 (1H dd, H-5′), 4.07 (1H t, $J_{14,15a}=J_{15a,15b}$ 10.5 Hz, H-15), 4.29 (1H d, H-1′), 4.37 (1H ,H-9), 4.38 (1H dd, $J_{14,15b}$ 6 Hz, H-15b), 4.61 (1H dd, J 4 and 7.5 Hz, H-18), 5.35 (1H slightly br d, $J_{13,14}$ 10 Hz, H-13), 5.67 (1H dd, $J_{9,10}$ 3.5 Hz, $J_{10,11}$ 15.5 Hz, H-10), 6.34 (1H dd, $J_{9,11}\simeq 1.5$ Hz, H-11).

Irradiation of H-14 caused the signals of H-15a and -15b to collapse to two doublets, and the doublet of H-13 to a singlet. Irradiation of H-9 collapsed the double doublets of H-10 and those of H-11 to a doublet, respectively, to form an AB quartet.

*Deacetalation of **14** (to Give **16**).* To a solution of **14** (46.6 mg) in acetonitrile (1.4 ml) was added gradually 0.1 M aqueous hydrochloric acid (1.4 ml) and the mixture was kept at room temperature for 1 h. After addition of aqueous saturated sodium hydrogencarbonate (1.5 ml), the mixture was extracted with chloroform. The chloroform-soluble products were chromatographed with silica gel with chloroform–methanol–28% aqueous ammonia (9 : 1 : 0.1) to give an amorphous solid of **16**, 32.3 mg (78%), $[\alpha]_D^{25}$ −12° (*c* 0.5, chloroform), UV λ_{max} (MeOH) 236 nm (log ε 4.34).

Found: C, 61.83; H, 8.81; N, 2.07%. Calcd for $C_{31}H_{53}NO_{10}$: C, 62.08; H, 8.91; N, 2.34%.

^{1}H-NMR ($CDCl_3$): δ 5.43 (1H d, H-13), 5.50 (1H dd, H-10), 6.21 (1H d, H-11), 9.81 (1H s, -CHO); $J_{9,10}$ 9, $J_{10,11}$ 16, $J_{13,14}$ 10 Hz.

*Oxidation of **15** (to Give **18**).* To an ice-cold solution of **15** (73.1 mg) in tetrahydrofuran (1.5 ml) was added DDQ (49.3 mg) and the solution was kept at room temperature for 1 h. The solution showed, on TLC with chloroform–methanol–28% aqueous ammonia (10 : 1 : 0.1), spots of R_f 0.4 (**18**), 0.33 (**15**), 0.1 and 0. Concentration gave a residue which was charged on a silica-gel column (7 g) and the column was washed with ethyl acetate (350 ml). DDQ and the reduced compound of DDQ were eluted. The developing solvent was then changed to chloroform–methanol–28% aqueous ammonia (12 : 1 : 0.1) to elute **18** to give a pale-brown solid (59.5 mg). The methanol solution of the solid was decolorized with charcoal and, after evaporation, the residue was chromatographed with chloroform–methanol–28% aqueous ammonia (15 : 1 : 0.1) to give a colorless solid of **18**, 34.4 mg (47%); recrystallization from acetone–hexane gave plates, mp 165—168 °C, $[\alpha]_D^{18}$ +16° (*c* 0.5, chloroform); UV λ_{max} (MeOH) 284 nm (log ε 4.32); IR(KBr): 1590, 1680, 1730 cm^{-1}; *m/e* 671 (M$^+$).

Found: C, 61.56; H, 9.06; N, 2.01%. Calcd for $C_{35}H_{61}NO_{11}\cdot 1/2\,H_2O$: C, 61.74; H, 9.18; N, 2.06%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 1.83 (3H d, $J\simeq 1$ Hz, Me(20)], 2.50 (6H s, NMe_2), 4.18 (1H t, H-15a), 4.30 (1H d, H-1′), 4.46 (1H dd, H-15b), 4.66 (1H m, H-18), 5.85 (1H d, H-13), 6.35 (1H d, H-10), 7.17 (1H d, H-11); $J_{10,11}$ 15, $J_{13,14}$ 11, $J_{14,15a}$ 10, $J_{14,15b}$ 6.5, $J_{15a,15b}$ 10 Hz.

*Deacetalation of **18** (to Give **19**).* Compound **18** was treated similarly as described for **16** to give crude **19** in *ca.* 80% yield (the purity was estimated to be *ca.* 90%). Repeated column-chromatography gave no pure product. On TLC with chloroform–methanol–28% aqueous ammonia (10 : 1 : 0.1), it gave the spots of R_f 0.3 (**19**) and 0.24 (slight).

^{1}H-NMR (in $CDCl_3$ at 250 MHz; peaks for **19** were only cited): δ 4.24 (1H d, H-1′), 4.27 (1H dd, H-15b), 4.48 (1H t, H-15a), 5.88 (1H d, H-13), 6.21 (1H d, H-10), 7.18 (1H d, H-11), 9.80 (1H s, -CHO); $J_{10,11}$ 15.5, $J_{13,14}$ 10.5, $J_{14,15a}$ 10, $J_{14,15b}$ 6, $J_{15a,15b}$ 10 Hz.

*3,21,2′,4′-Tetra-O-acetyl Derivative (**20**) of **18**.* A solution of **18** (82.9 mg) in pyridine (0.8 ml) containing acetic anhydride (0.21 ml) was kept at 50 °C for 2 d. Usual work-up gave amorphous solid of **20**, 92 mg (89%), $[\alpha]_D^{22}$ +65° (*c* 1, chloroform).

Found: C, 61.72; H, 8.12; N, 1.75%. Calcd for $C_{43}H_{69}NO_{15}$: C, 61.48; H, 8.28; N, 1.67%.

^{1}H-NMR ($CDCl_3$): δ 1.90, 2.05, 2.10 and 2.15 (each 3H s, Ac).

*3,21-Di-O-acetyl Derivative (**21**) of **18**.* A methanol solution (2.0 ml) of **20** (41.6 mg) was kept at 50 °C overnight. Evaporation gave a residue, which was chromatographed on a silica-gel column with chloroform–methanol–28% aqueous ammonia (25 : 1 : 0.1) to give an amorphous solid, 37.2 mg (99%), $[\alpha]_D^{23}$ +61° (*c* 1, chloroform): TLC: R_f 0.32 (*cf.* **17**: R_f 1) (20 : 1 : 0.1 chloroform–methanol–28% aqueous ammonia).

Found: C, 61.68; H, 8.50; N, 1.76%. Calcd for $C_{39}H_{65}NO_{13}$: C, 61.97; H, 8.64; N, 1.85%.

^{1}H-NMR (in $CDCl_3$ at 250 MHz): δ 0.89 (3H t, H-23), 1.10 [3H d, J 7 Hz, Me(16)], 1.11—1.24 [9H, Me(19) and $(OCH_2C\underline{H}_3)_2$], 1.31 [3H d, Me(6′)], 1.57 (2H m, H-22), 1.92 [3H very narrow m, Me(20)], 2.04 and 2.11 (each 3H s, 3- and 21-*O*-Ac), 2.37 (1H t, J 10 Hz, H-3′), 2.51 (6H s, NMe_2), 3.04 (1H t, $J\simeq 10$ Hz, H-4′), 3.12 (1H m, H-14), 3.30 (1H m, H-5′), 3.55 (1H dd, H-2′), 3.94 (1H dd, $J_{14,15a}$ 5 Hz, $J_{15a,15b}$ 10.5 Hz, H-15a), 4.29 (1H d, $J_{1',2'}$ 7.5 Hz, H-1′), 4.48 (1H t, J 10 Hz, H-15b), 4.59 (1H dd, J 3.7 and 7.5 Hz, H-18), 4.86 (1H br m, H-3), 4.96 (1H m, H-21), 5.77 (1H slightly br d, $J_{13,14}$ 10 Hz, H-13), 6.40 (1H sharp d, $J_{10,11}$ 15.5 Hz, H-10), 7.16 (1H slightly br d, H-11).

Irradiation of H-14 collapsed the doublet of H-13 to a singlet, the quartet of H-15a and the triplet of H-15b to an AB quartet in total, and the multiplet of H-21 to a quartet. Irradiation at δ 1.57 (H-22a,b) collapsed the triplet of H-23 to a singlet.

*Deacetalation of **21** (to Give **22**).* Treatment of **21** similarly as described for **16** gave an amorphous solid of **22** in 79% yield, $[\alpha]_D^{22}$ +61° (*c* 1, chloroform).

Found: C, 61.67; H, 8.12; N, 1.98%. Calcd for $C_{35}H_{55}NO_{12}$: C, 61.66; H, 8.13; N, 2.05%.

We are grateful to Professor Hamao Umezawa of Institute of Microbial Chemistry for his support and encouragement. We also wish to thank Mr. Saburo Nakada, Department of Applied Chemistry, Keio University, for the microanalysis, and Dr. Masa Hamada of Institute of Microbial Chemistry, for the bioassay. Thanks are also due to Miss Miyako Igarashi of our Institute for her helpful assistance to make the manuscript.

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