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Chemical Transformation of Tylosin, a 16-Membered Macrolide, and Its Structure-Activity Relationship

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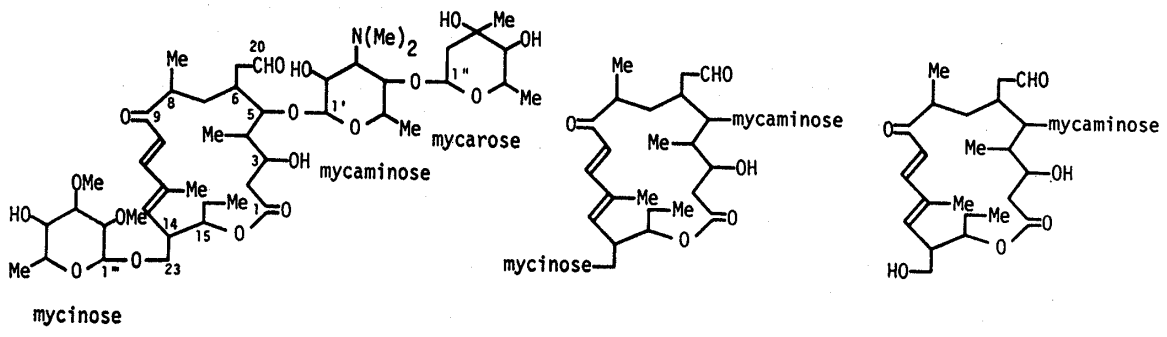
Tylosin is a 16-membered macrolide antibiotic used as veterinary antimicrobial agent. In this paper, we describe the structure of a novel condensation product obtained by acidic hydrolysis and alkaline treatment of tylosin, as well as the isolation of the aglycone part, tylonolide 5,20-hemiacetal, by application of a modified Polonovski reaction to tylosin. Furthermore, we describe syntheses of the intermediates of tylosin biosynthesis and of related compounds, and also the relationship between the structure and antimicrobial activity.

Keywords——tylosin; chemical transformation; biosynthetic intermediate; structure–activity correlation; macrolide

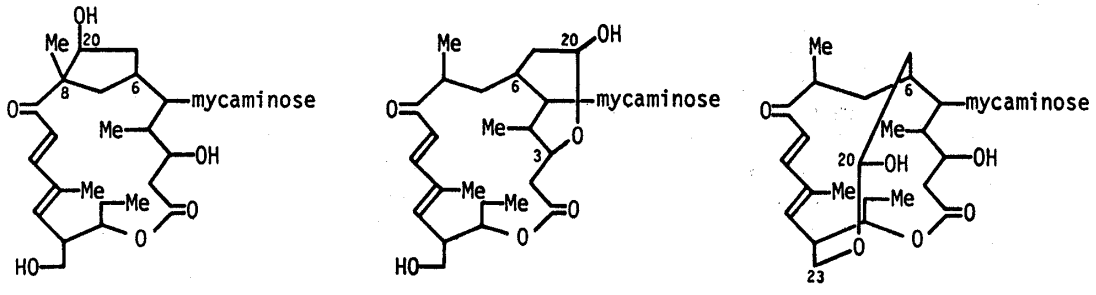
The Structure of a Novel Acidic Degradation Product of Tylosin

Tylosin (**1**) possesses three sugars, namely, mycarosyl mycaminose linked glycosidically at the 5-position and mycinose linked *via* the hydroxymethyl group at the 14-position of the 16-membered lactone ring.[1,2] It is well known that mild acidic hydrolysis of **1** causes the removal of mycarose to produce demycarosyltylosin (**2**). More vigorous hydrolysis[2] yields 5-*O*-mycaminosyltylonolide (**3**) accompanined with removal of mycinose, as shown in Chart 1.



1 **2** **3**

Chart 1



4a **4b** **4c**

Chart 2

By vigorous acidic hydrolysis of **1**, we obtained an unusual bicyclic lactone compound (**4**) in 10% yield. The molecular formula $C_{31}H_{51}NO_{10}$ deduced from the high resolution mass, M^+ m/z 597 (Found, 597.349; Calcd for $C_{31}H_{51}NO_{10}$, 597.351), and the fragment peaks at m/z 407 (aglycone), 190 and 174 (mycaminose) are identical with those of **3**, indicating that compound **4** is a structural isomer of **3**. A blue shift of about 7 nm in the ultra violet (UV) absorption

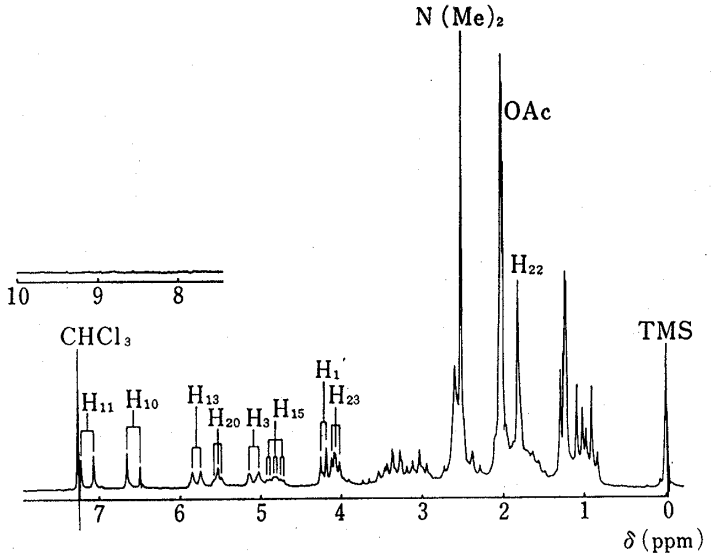


Fig. 1. 100 MHz ^{1}H-NMR Spectrum of 3,20,23-Tri-*O*-acetyl-8,20-cyclo-20-hydroxy-5-*O*-mycaminosyltylonolide (**6**)

TABLE I. ^{1}H-NMR Chemical Shifts and Coupling Constants of 8,20-Cyclo-20-hydroxy-5-*O*-mycaminosyltylonolide (**4**) and Its Triacetate (**6**)

Protons	**4**		**6**	
	Chem. shift δ (ppm)	Coupling constants (Hz)	Chem. shift δ (ppm)	Coupling constants (Hz)
C_2–H	*ca.* 1.3		2.65 br	
C_3–H	*ca.* 3.1		5.08 br d	$J_{2,3}=9.0$
C_4–H	*ca.* 3.2		*ca.* 1.7	
C_5–H	*ca.* 1.7		*ca.* 3.3	
C_6–H	1.5—2.0		1.5—2.0	
C_7–H	2.0—3.0		2.0—3.0	
C_8–H	2.5—3.0		2.0—3.0	
C_{10}–H	6.58 d	$J_{10,11}=15.0$	6.58 d	$J_{10,11}=15.0$
C_{11}–H	6.97 d	$J_{10,11}=15.0$	7.14 d	$J_{10,11}=15.0$
C_{13}–H	5.76 br d	$J_{13,14}=10.0$	5.80 br d	$J_{13,14}=10.0$
C_{14}–H	2.90 br		3.03 t	$J_{13,14}=10.0$
C_{15}–H	4.98 d t		4.82 d t	
C_{16}–H	1.55 br		1.68 br	
C_{17}–H	0.92 t	$J_{16,17}=6.0$	0.93 t	$J_{16,17}=6.0$
C_{18}–H	1.03 d	$J_{4,18}=8.0$	1.07 d	$J_{4,18}=8.0$
C_{19}–H	1.95 br		2.10 br	
C_{20}–H	4.59 br t		5.52 br t	
C_{21}–H	1.27 s		1.22 s	
C_{22}–H	1.81 s		1.81 s	
C_{23}–H	3.69 br d		4.07 t	$J_{14,23}=4.0$
–OH	2.70 br		—	
$-COCH_3$	—		2.01 s	

of **4** [λ_{max}^{MeOH} 283.5 nm (ε, 19500) in **3** and λ_{max}^{MeOH} 276.0 nm (ε, 19000) in **4**] suggests that the $\alpha,\beta,\gamma,\delta$-unsaturated ketone chromophore moiety in **4** is subject to steric torsion inherent in the formation of a bicyclic lactone. In the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **4**, the aldehyde proton signal observed at δ 9.7 in **3** was absent and the signal of a proton attached to a newly formed oxycarbon was observed at δ 4.59 as a broad triplet. The other proton signals on the aglycone moiety in **4** showed almost the same chemical shift values as those of **3**. These spectral data led to three possible structures, **4a**, **4b** and **4c**, for **4**, as shown in Chart 2. Acetylation of **4** with acetic anhydride in pyridine afforded the pentaacetate (**5**), M^+ m/z 807, whose mass and ^{1}H-NMR spectra indicated the introduction of three acetyl groups on the aglycone moiety, in addition to acetylation of two hydroxyl groups on mycaminose. Solvolysis of **5** in methanol resulted in the removal of the two acetyl groups on the mycaminose moiety, to afford the triacetate **6**, $C_{37}H_{57}NO_{13}$, M^+ m/z 723, fragment peaks; m/z 533 (triacetyl aglycone), m/z 174 (mycaminose). The spectral data described above indicated the presence of a hydroxyl group newly formed by acidic hydrolysis besides two hydroxyl groups at the 3- and 23-positions on the lactone ring. Therefore, the structure **4a** seems probable for **4**. In the ^{1}H-NMR spectrum (Fig. 1) of **6**, the signal of the hydroxymethyl group at the 23-position that appeared as a doublet at δ 3.69 in **4** was observed to be shifted to δ 4.07 by the introduction of an acetyl group at this position. This downfield shift means that the primary hydroxyl group must be located at the 23-position in compound **4** as well as in **3**. The observation that the proton (δ 5.08, J=9.0 Hz) at the base of a newly introduced acetoxyl group in **6** couples with the methylenic proton at the 2-position indicates the presence of a hydroxyl group at the 3-position in **4**. The methyl group substituted at the 8-position, which is observed as a doublet at δ 1.16 in **3**, appeared as a singlet at δ 1.27 in **4** and at δ 1.22 in **6**. In addition to the spectral evidence described above, the proton at the base of the newly appeared hydroxyl, seen as a broad triplet at δ 4.59 in **4**, was observed to shift downfield to δ 5.52 in the acetate **6**, suggesting the possibility of a 6,8-bicyclic structure condensed between the formyl group and the methine carbon at the 8-position. The validity of the structure **4a** was also confirmed by comparison of the ^{13}C-NMR spectral data for compounds **1** and **4**, as indicated in Chart 4. The ^{13}C-NMR spectrum of **4** revealed the conversion of the formyl carbon signal in **1** to the oxycarbon signal at δ 73.7 (off-resonance; doublet). The C-8 methine carbon observed as a doublet in the off-resonance spectrum at δ 40.3 in **1** appeared as a singlet (off-resonance) at δ 36.8 in **4**. Comparison of the chemical shift of the ketone carbonyl at the 9-position in **1** and **4**

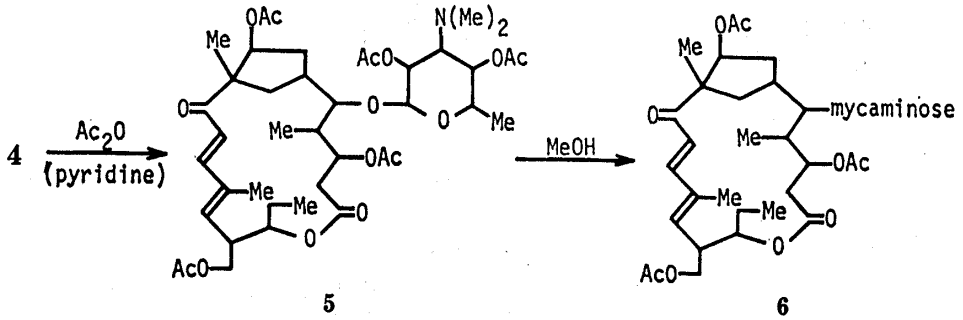


Chart 3

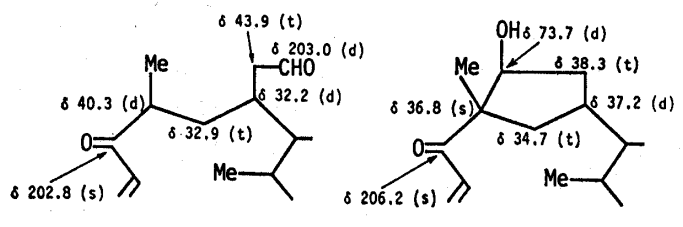


Chart 4

revealed a downfield shift of 3.4 ppm in **4** (δ 202.8 in **1** and 206.2 in **4**) based on the substitution effect at the C-8 methine carbon. The structure of **4** was assigned as 8,20-cyclo-20-hydroxy-5-*O*-mycaminosyltylonolide (**4a**). On the other hand, Suzuki *et al.*[3] obtained a new condensate by heating B-58941, a 16-membered macrolide, under reflux in methanol with a sulfonic acid-type ion exchange resin, and they reported that aldol condensation took place between the C-8 methine and formyl carbons. The formation of **4** also seems to proceed *via* aldol condensation.

Isolation of Tylonolide 5,20-Hemiacetal, a Tylosin Aglycone

In the course of studying the structure-microbial activity correlation of 16-membered macrolides, we have reported the effective isolation of the aglycone moiety from leucomycin A_3 by application of the modified Polonovski reaction to leucomycin A_3 *N*-oxide.[4] In this paper, we describe the application of the reaction to tylosin to isolate the aglycone moiety of tylosin (**1**). 5-*O*-Mycaminosyltylonolide (**3**) obtained by acid hydrolysis of **1** was converted to its *N*-oxide **7** by treatment with *m*-chloroperbenzoic acid in chloroform, in high yield. The modified Polonovski reaction of **7** with acetic anhydride in chloroform afforded compound **8**, $C_{23}H_{36}O_7$, M^+ *m/z* 424, in *ca.* 10% yield.

In the ^{1}H-NMR spectrum of **8**, the signals of a formyl group and a dimethylamino group on the mycaminose moiety observed at δ 9.75 and δ 2.6, respectively, in **3** disappeared in **8**, indicating that **8** is an aglycone moiety. The proton signal seen as a triplet at δ 5.5 in **8** can be assigned to the oxyproton at C-20 since the signal changes to a singlet upon irradiation of the methylenic protons at the 19-position. From the fact that acetylation of **8** with acetic anhydride in pyridine affords the triacetate, in addition to the result described above, compound **8** could be assigned as tylonolide 5,20-hemiacetal, which has a 5-membered hemiacetal ring formed between the formyl group and the hydroxyl group at the 5-position produced by removal of the mycaminose moiety (Chart 5).

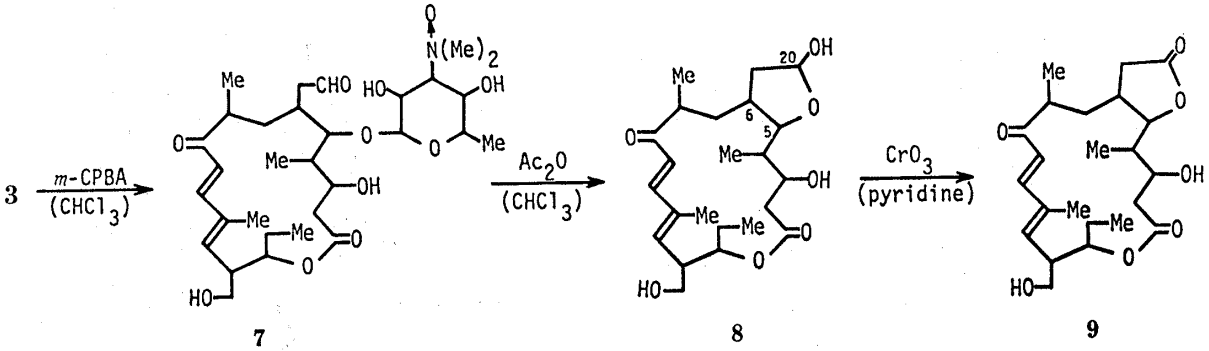


Chart 5

Oxidation of compound **8** with CrO_3 in pyridine gave its dilactone **9**. The high resolution EI-mass spectrum of **9** afforded the molecular formula $C_{23}H_{34}O_7$ (M^+ *m/z* 422), and the IR spectrum exhibited a characteristic absorption band for an ester carbonyl of a 5-membered lactone at 1770 cm^{-1}. In the ^{1}H-NMR spectrum (Fig. 2) of **9**, the fact that irradiation of the methylenic protons (C-2 position) at δ 2.5 collapsed the proton observed as a broad doublet at δ 3.6 (J=11.1 Hz) to a singlet indicated that the proton could be assigned as that at the base of the hydroxyl at the C-3 position. The proton signal at δ 4.4 (dd, J=9.0 Hz and 4.8 Hz) could be assigned to the C-5 proton since it changes to a doublet (J=9.0 Hz) on irradiation of the C-6 methine proton at δ 2.2. The chemical shift values and coupling constants in the ^{1}H-NMR spectrum of **9** are very similar to those of 9-dehydroleuconolide A_3 dilactone (**10**) which was obtained by CrO_3 oxidation of leuconolide A_3 5,18-hemiacetal.[4] The CD spectral evidence in addition to NMR spectral data for **9** and **10** indicated that the aglycones

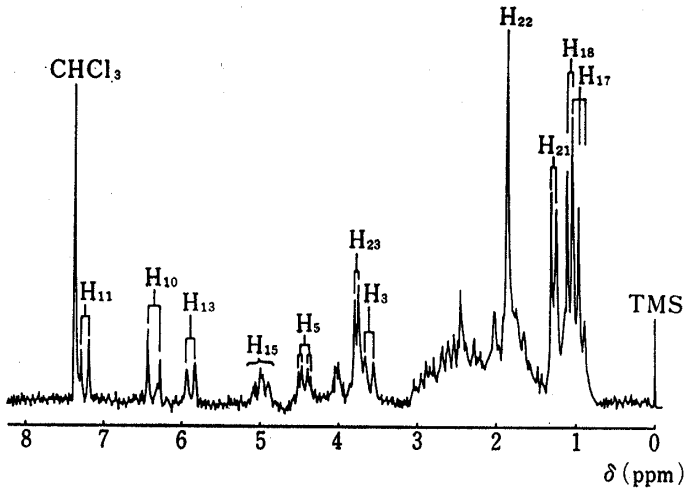


Fig. 2. 100 MHz ^{1}H-NMR Spectrum of Tylonolide Dilactone (**9**)

take similar conformations, as reported in a previous paper.[6] The configuration and conformation of **9** will be described in detail elsewhere in connection with those of **1** and related compounds.

On the other hand, in examining the structure–activity correlation of tylosin, we were interested in the microbial activity of 23-*O*-mycinosyltylonolide-5,20-hemiacetal (**12**) in which only mycinose is glycosidically linked to the aglycone portion. Compound **12** was prepared by application of the modified Polonovski reaction of tylosin *N*-oxide (**11**) in a manner similar to that used for the isolation of **8**. The structural assignment of **12** was confirmed from the mass [*m/z* 698 (M^+), 407 (aglycone), 191 (mycinose)] and ^{1}H-NMR (two methoxyls at C-2 and C-3 positions on mycinose at δ 3.55 and 3.62, respectively; anomeric proton of mycinose at δ 4.55; C-20 methine proton at δ 5.50) spectral data.

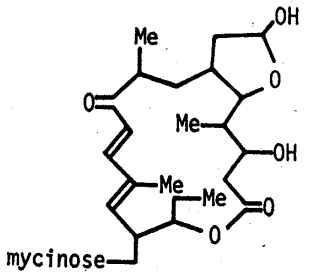


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Chart 6

The Structure of the Bicyclo Lactone obtained by Alkali Treatment of Tylosin

With regard to 16-membered macrolides belonging to the lecuomycin group, we have reported the structure of a bicyclo lactone formed by condensation between the C-3 position and the methylene adjacent to the formyl group on the lactone ring under mild alkaline conditions.[8] This reactivity indicates that the 3-position and the methylene are in close proximity within the lactone ring.

In examining the configurational and conformational similarity of leucomycin A_3 and tylosin, we applied this reaction to tylosin. Condensation between the 3-position and the methylene at the 19-position under alkaline conditions in leucomycin A_3 may require the presence of an acetoxyl group at the 3-position in view of the reaction mechanism.[8] Thus, **1** was acetylated with acetic anhydride in pyridine to obtain the tetraacetate (**13**). The acetate was subjected to solvolysis in methanol, affording the triacetate (**14**), in which the acetoxyl group at the 2-position on the mycaminose moiety is eliminated. The resulting triacetate (**14**) was heated under reflux with $LiOH \cdot H_2O$ in ethanol to give **15** in 70% yield. The mass spectrum of **15** showed a molecular ion peak, *m/z* 897, and fragment peaks *m/z* 145, 191 and 174 arising from mycarose, mycinose and mycaminose moieties, respectively.

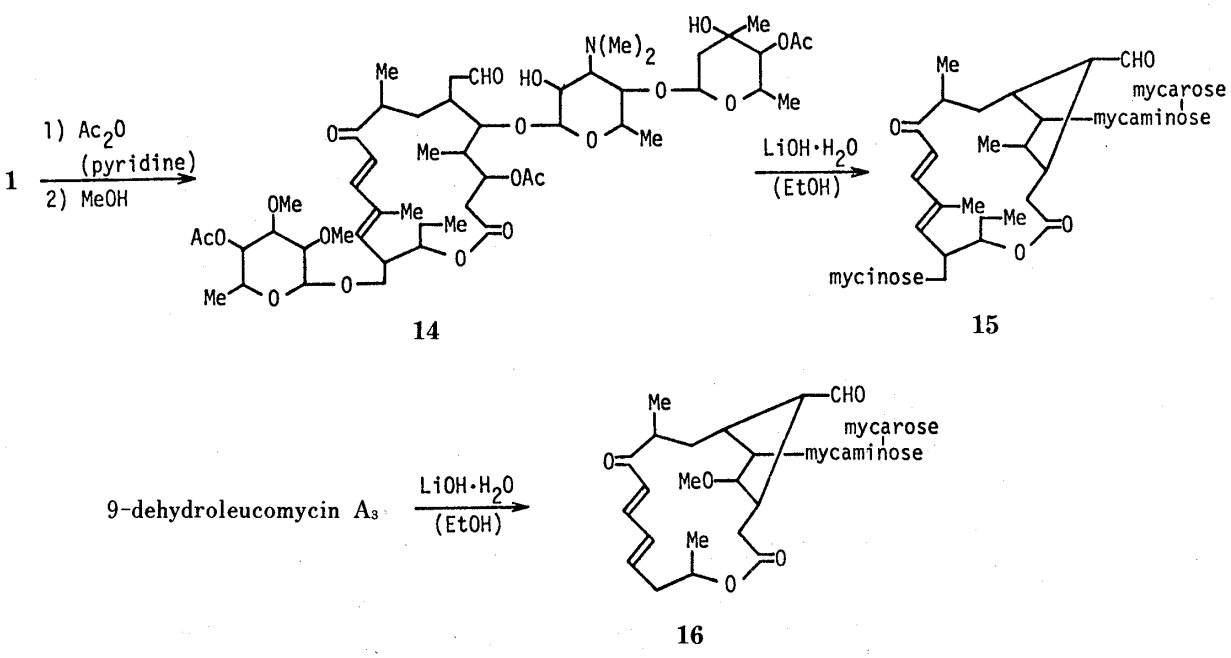

Chart 7

As judged from the ^{1}H-NMR spectrum of **15**, three acetoxyl groups present at the 3-, 4''- and 4'''-positions in **14** are eliminated in **15** by alkaline treatment. The aldehyde proton signal seen as a broad singlet at δ 9.75 in **1** was observed as a doublet ($J_{19,20}$=3.5 Hz) at δ 9.75 in **15**. This signal changed to a singlet on irradiation of the methine proton at δ 2.60, indicating that the methine proton must be located at the vicinal position with respect to the aldehyde group. Observation of the fragment peaks at *m/z* 391 (aglycone) and *m/z* 563 corresponding to the structure with glycosidic linkage of mycinose to the aglycone in **15**, in addition to the ^{1}H-NMR spectral evidence described above, have led us to propose the structure 3-deoxy-3,6-bicyclo-tylosin for **15** (Chart 7). This reactivity of **1** and the observation of the same Cotton effects (215 nm ([θ] −19400 in **16**, [θ] −28400 in **15**), 279 nm ([θ] −8940 in **16**, [θ] −12260 in **15**), 333 nm ([θ] +7890 in **16**), 337 nm ([θ] +10270 in **15**)) in the CD curves[6] of compound **15** and 3-deacetoxy-3,6-bicyclo-9-dehydroleucomycin A_3 (**16**) derived from 9-dehydroleucomycin A_3 and tylosin reveal that the acetoxyl group at C-3 and the aldehyde group are conformationally in close proximity and further that both aglycones have similar conformation.

The Syntheses of Intermediates in Tylosin Biosynthesis and Related Compounds

It has been clarified that the aglycone moiety of tylosin is built up from two acetates, five propionates and one butyrate by the use of ^{13}C labeled biosynthetic precursors and ^{13}C-NMR spectrometry.[9] Recently, we isolated compound **17**, designated protylonolide,[10] from the culture broth of mutant No. 261 obtained by NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) treatment of a tylosin-producing strain *Streptomyces fradiae* KA-427. Protylonolide (**17**), which is a direct precursor of tylosin, differs structurally from the aglycone moiety of tylosin in that the aldehyde at the 20-position and the hydroxymethyl group at the 23-position have been reduced to methyl groups. Tylosin-related compounds that were considered to be possible biosynthetic intermediates were synthesized for investigation of the biosynthetic pathway from protylonolide to tylosin involving the order of oxidation of the methyl groups

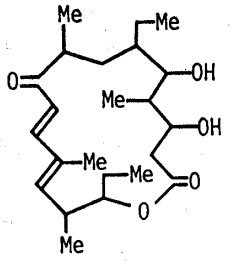

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Chart 8

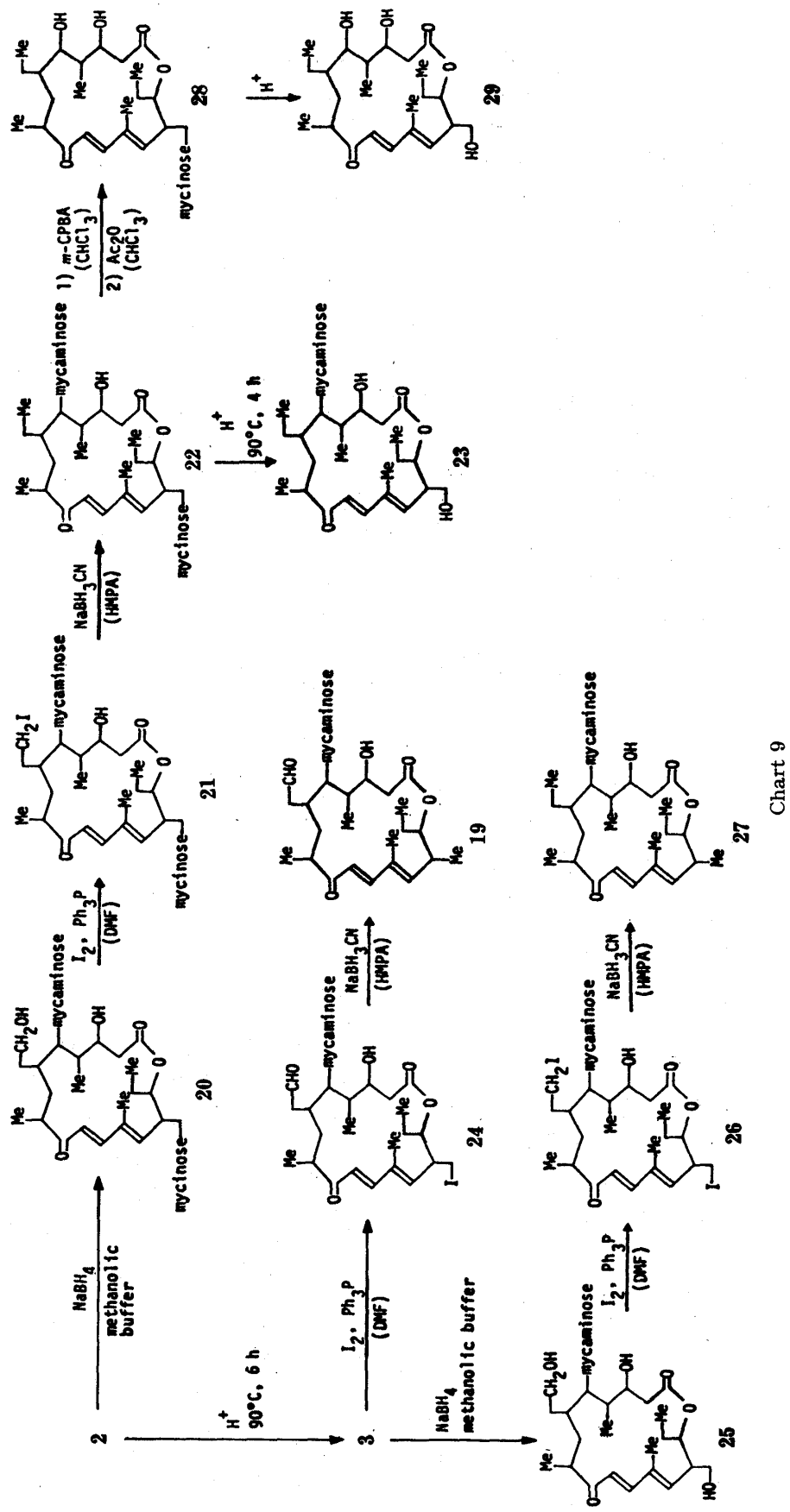

Chart 9

at the 20- and 23-positions and of addition of mycarose, mycaminose and mycinose moieties to the aglycone. As shown in Chart 9, in this section, we describe the reductive methylation of the formyl group at the 20-position and of the hydroxymethyl group at the 23-position formed by removal of mycinose from tylosin and related compounds, and also the chemical transformation from tylosin to deepoxycirramycin A_1 (**19**), which was also obtained by reduction of cirramycin A_1 (**18**) with KI in acetic acid.

The $NaBH_4$ reduction of **2** in methanolic-phosphate buffer solution afforded 20-dihydrodemycarosyltylosin (demycarosylrelomycin) (**20**) in which only the formyl group at the 20-position has been reduced to a primary alcohol. The iodination of **20** in the presence of iodine and triphenylphosphine in dimethylformamide affords an iodinated compound **21** [mp 102—104°C, *m/z* 883 (M^+), 756 ($M^+ - I$), 507 (aglycone)]. With regard to iodination of the primary alcohol in **20**, we also carried out the tosylation of the alcohol with *p*-toluenesulfonyl chloride, followed by iodination of the tosylated product with NaI in acetone. However, since the former reaction is superior to the latter in yield, the former reaction was applied for the iodination of **20**. The iodinated compound **21** was then reduced with $NaBH_3CN$ in hexamethylphosphoric triamide to give 20-deoxydemycarosylrelomycin (**22**) [mp 96—97°C, *m/z* 757 (M^+), 583 (M^+ —mycaminose), 392 (aglycone)] in 84% yield. The structural assignment of compounds **21** and **22** were easily deduced from mass and NMR spectral data. Removal of the mycinose moiety by acidic hydrolysis of **22** gave 20-deoxy-5-*O*-mycaminosylrelonolide (**23**) [mp 109—112°C, $C_{31}H_{53}NO_9$ *m/z* 583 (M^+), 939 (aglycone)]. In a similar manner, iodination of 5-*O*-mycaminosyltylonolide (**3**) with iodine and triphenylphospine afforded 23-deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**24**) [mp 116—117°C, *m/z* 707 (M^+), 580 ($M^+ - I$), 517 (aglycone)], which was then reduced with $NaBH_3CN$ to deepoxycirramycin A_1 (**19**) [mp 107—109°C, *m/z* 581 (M^+), ($C_{31}H_{51}NO_9$), UV λ_{max}^{MeOH} 283 nm (ε, 16000)]. The diagnostic fragmentation pattern of **24** is consistent with its structure, as shown in Fig. 3. The structure of **19** was confirmed by the identity of both IR and mass spectral data with those of a compound obtained by deepoxidation of cirramycin A_1 (**18**) with KI in acetic acid.[11] On the other hand, selective reduction of **3** with $NaBH_4$ in methanolic-phosphate buffer gave 5-*O*-mycaminosylrelonolide (**25**), mp. 108—111°C, which was then iodinated to obtain 20,23-dideoxy-20,23-diiodo-5-*O*-mycaminosylrelonolide (**26**) [mp 105—106°C, *m/z* 819 (M^+), 692 ($M^+ - I$), 565 ($M^+ - 2I$), 629 (aglycone), 375 (aglycone—2I)]. The compound **26** was converted by $NaBH_3CN$ reduction to 5-*O*-mycaminosylprotylonolide (**27**) [mp 95—96°C, $[\alpha]_D^{24}$=14.1°, λ_{max}^{MeOH} 283 nm (ε, 23000), $C_{31}H_{53}NO_8$, *m/z* 567.377 (M^+)]. It is well known that the carbon substituted with iodine resonates at high field. The ^{13}C-NMR chemical shift assignments for compounds **3**, **19**, **24**, **26** and **27** are listed in Table II. The signal of the C-23 carbon seen at δ 62.6 in **3** showed a remarkable shift to the high field region at δ 5.2 as a result of introduction of iodine at the C-23 position in **24**. The ^{13}C-NMR spectrum of **27** obtained by $NaBH_3CN$ reduction of **26** reflected the conversion of the iodinated methyl at the 23-position to a methyl group (δ 12.1).

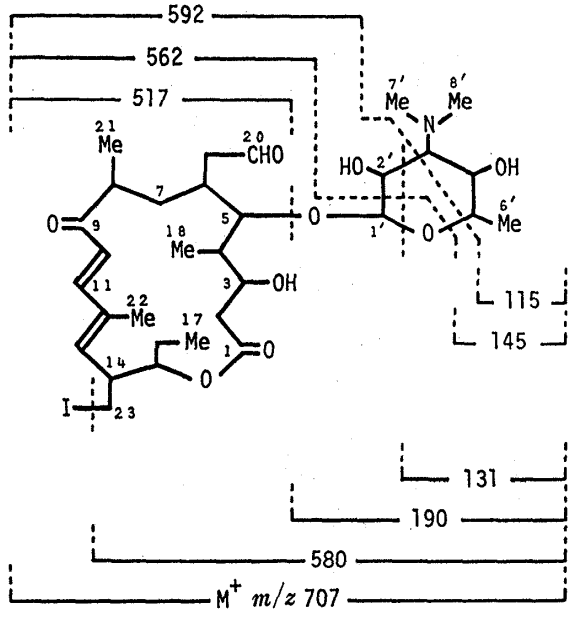

Fig. 3. Diagnostic Fragmentations of 23-Deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**24**)

On the other hand, in order to investigate the possibility of biosynthesis of tylosin *via*

TABLE II. ^{13}C-NMR Chemical Shifts of 5-*O*-Mycaminosyltylonolide (**3**), 23-Deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**24**), Deepoxycirramycin A_1 (**19**), 20,23-Dideoxy-20,23-diiodo-5-*O*-mycaminosylrelonolide (**26**) and 5-*O*-Mycaminosylprotylonolide (**27**)[a]

Carbon No.	Chemical shift δ(ppm)				
	3	**24**	**19**	**26**	**27**
C-1	173.9 s[b]	173.6 s	173.9 s	174.1 s	174.7 s
C-2	39.6 t	39.5 t	39.4 t	39.6 t	39.7 t
C-3	70.9 d	70.6 d	70.8 d	71.0 d	71.0 d
C-4	44.7 d	44.8 d	44.8 d	44.6 d	44.2 d
C-5	81.1 d	81.1 d	81.1 d	78.9 d	84.3 d
C-6	31.9 d	31.9 d	31.7 d	41.7 d	41.7 d
C-7	32.7 t	32.6 t	32.6 t	33.1 t	34.0 t
C-8	40.5 d	40.5 d	40.5 d	40.2 d	40.5 d
C-9	203.7 s	203.1 s	203.3 s	203.2 s	204.1 s
C-10	118.7 d	119.2 d	118.4 d	119.5 d	118.7 d
C-11	148.2 d	147.4 d	148.2 d	147.1 d	147.4 d
C-12	135.7 s	135.6 s	133.3 s	135.7 s	133.5 s
C-13	142.2 d	142.6 d	146.0 d	142.3 d	144.9 d
C-14	47.1 d	45.7 d	38.8 d	45.5 d	38.7 d
C-15	73.3 d	77.3 d	78.9 d	77.1 d	78.0 d
C-16	25.5 t	24.9 t	24.7 t	24.9 t	24.8 t
C-17	9.0 q	9.0 q	9.0 q	9.5 q	9.2 q
C-18	9.7 q	9.5 q	9.6 q	9.5 q	9.6 q
C-19	42.5 t	43.7 t	43.7 t	32.9 t	21.1 t
C-20	203.2 d	203.1 d	203.1 d	9.3 t	16.3 q
C-21	17.4 q	17.4 q	17.5 q	17.5 q	17.3 q
C-22	13.1 q	13.2 q	12.9 q	13.3 q	12.9 q
C-23	62.2 t	5.2 t	12.9 q	5.3 t	12.1 q

a) Chemical shifts for the carbons of sugar moieties are omitted for convenience.
b) Multiplicities in the off-resonance spectrum: s, singlet; d, doublet; t, triplet; q, quartet.

20-deoxy-23-*O*-mycinosylrelonolide (**28**) from protylonolide, compound **28** was prepared by application of the modified Polonovski reaction to the *N*-oxide of 20-deoxydemycarosylrelomycin (**22**), as shown in Chart 9. Heating of the *N*-oxide with acetic anhydride in chloroform under reflux gave 20-deoxy-23-*O*-mycinosylrelonolide (**28**) in 80% yield. Compound **28** was heated under reflux with 0.05 N HCl for 23 h to yield 23-hydroxyprotylonolide (**29**) as a white powder. The ^{1}H-NMR spectral assignments of the derivatives **28** and **29** are in good accord with their structures. The derivatives thus obtained were used in feeding experiments for biosynthetic studies of **1**; the results will be reported later.

Structure-activity Correlation of Tylosin and Related Compounds

There are some reports on the antibacterial activities of tylosin acyl derivatives and tylosin-type antibiotics, but no work on the relationship between chemical structure and antimicrobial activity has been reported. Thus, the antibacterial activity of the tylosin-related compounds described above was tested. The MIC (minimum inhibitory concentration; μg/ml) values against a variety of microorganisms were determined by the agar dilution method (Table III). As can be seen from Table III, demycarosyltylosin (**2**) showed almost the same antimicrobial activity as **1**, indicating that the presence of mycarose is not essential for the appearance of antimicrobial activity. On the other hand, 5-*O*-mycaminosyltylonolide (**3**) in which the mycinose moiety is absent, showed higher activity against Gram-negative bacteria such as *E. coli* and *K. pneumoniae*. It should be noted that 23-deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**24**) exhibited potent antibacterial activity, as did erythromycins (**32**). The MIC of **24** was less than 0.025 μg/ml against *B. subtilis* PCI 219 and *Sar. lutea* PCI 1001 and

Table III. Antibacterial Activities of Leucomycin A_3 (31), Erythromycins (32), Tylosin (1) and Related Compounds

Compd.	MIC (μg/ml)							
	Staph. aureus FDA 209P	*B. subtilis* PCI 219	*Sarc. lutea* PCI 1001	*E. coli* NIHJ	*K. pneumoniae*	*Salm. Typhimurium*	*P. vulgaris* IFO 3168	*M. smegmatis* ATCC 607
1	0.78	0.2	0.1	50	50	50	>100	25
2	0.4	0.4	<0.025	25	12.5	100	>100	>100
20	1.56	3.125	0.2	25	50	>100	>100	>100
21	0.05	0.1	<0.025	50	100	>100	>100	100
22	0.05	0.2	<0.025	50	25	>100	>100	25
23	6.25	25	6.25	25	25	100	>100	100
3	1.56	1.56	0.1	25	3.12	25	>100	>100
24	0.05	<0.025	<0.025	3.12	6.25	12.5	12.5	>100
19	0.4	0.4	0.1	1.56	3.12	6.25	25	>100
25	>100	>100	12.5	100	50	>100	>100	>100
26	1.56	1.56	0.78	50	>100	>100	100	25
27	3.12	1.25	1.56	25	25	>100	50	50
17	>100	>100	>100	>100	>100	>100	>100	>100
30	1.56	0.4	0.78	>100	>100	>100	>100	>100
31	0.4	0.2	<0.025	50	6.25	50	100	100
32	0.2	0.2	<0.025	6.25	6.25	12.5	100	50

24 exhibited the same activity as deepoxycirramycin A_1 (**19**) against *E. coli* NIHJ and *K. pneumoniae*. The significant activities of 5-*O*-mycaminosyltylonolide (**3**) and deepoxycirramycin A_1 (**19**) against Gram-negative bacteria indicate that the formyl group plays a significant role in the appearance of the activities. 20-Deoxydemycarosylrelomycin (**22**), which possesses a methyl group at the 20-position and a mycinose moiety linked at the hydroxymethyl group at the 23-position also showed significant activity (*Staph. aureus* FDA 209P; 0.05 μg/ml, *Sar. lutea* PCI 1001; 0.025 μg/ml), like 20-deoxy-20-iododemycarosylrelomycin (**21**). In the case of the derivatives in which the mycinose moiety is placed at the 23-position, the presence of a methyl group rather than a formyl group at the C-20 position tends to result in higher activity against Gram-positive bacteria.

On the other hand, compounds **8**, **9**, **10** and **20** arising from the aglycone moiety and the bicyclic lactones **4** and **15** showed no antimicrobial activity. It is likely that the reduction of activity caused by forming the bicyclic structure in compounds **4** and **15** is mainly due to the change of orientation of the aldehyde function in the molecule of **15** or to the change of conformation of the aglycone moiety. Furthermore, the marked reduction of antimicrobial activity for 23-*O*-mycinosyltylonolide 5,20-hemiacetal (**12**) and 20-deoxy-23-*O*-mycinosylrelonolide (**28**) is in accord with our finding[14] that the presence of the mycaminose moiety is important for the appearance of activity.

Experimental

Melting points were measured on a hot stage microscope and are uncorrected. The optical rotations were measured on a JASCO DIP-181 spectrometer. The UV spectra were measured on a Shimadzu UV-210A spectrometer. Mass spectra were taken with a JEOL JMS-D 100 spectrometer and high resolution mass spectra were taken with a JEOL JMS-01S spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL PS-100 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. Elemental analysis was done with a Perkin Elmer 240 machine.

8,20-Cyclo-20-hydroxy-5-*O*-mycaminosyltylonolide (4)——A solution of **1** (50 g) in 0.2N aq. HCl (1000 ml) was stirred for 4 h. The mixture was washed with chloroform (500 ml) to remove mycarose. The solution was adjusted to pH 8.0 and extracted with chloroform (700 ml×2). The extract was dried over anhydrous sodium sulfate and evaporated to dryness to give **2**. The product was dissolved in 0.5N HCl (1000 ml). The mixture was stirred at 90°C for 20 h, then washed with chloroform (500 ml) to remove mycinose, and the

aqueous layer was adjusted to pH 8.0 and extracted with chloroform (1000 ml×2). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. Purification of the residue was performed on a silica gel column and elution with $CHCl_3$–MeOH–conc. ammonia water (10: 1: 0.05) yielded **3** (6.5 g) and **4** (1.6 g) successively. Compound **4**: mp 98—99°C. UV λ_{max}^{MeOH} nm (ε): 276 (19000). MS *m/z*: 597 (M^+, 597.349; Calcd for $C_{31}H_{51}NO_{10}$: 597.351), 407 (aglycone), 174 (mycaminose). ^{1}H-NMR δ (ppm): 1.81 (3H, s, C-22), 2.50 (6H, s, $N(CH_3)_2$), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.98 (1H, d t, $J_{14,15}$=4.0 Hz, $J_{15,16}$=8.0 Hz, C-15), 5.76 (1H, br d, $J_{13,14}$=10.0 Hz, C-13), 6.58 (1H, d, J=15.0 Hz, C-10), 6.97 (1H, d, J=15.0 Hz, C-11).

5-*O*-Mycaminosyltylonolide *N*-Oxide (7)——A mixture of *m*-chloroperbenzoic acid (2.0 g) and compound **3** (3.7 g) in chloroform (7.0 ml) was stirred for 5 h under an argon atmosphere in an ice bath. The reaction mixture was then poured into 10% aq. sodium sulfite. The chloroform layer was dried over anhydrous sodium sulfate and removal of the solvent afforded the *N*-oxide (**7**, 2.6 g) as a white powder.

Tylonolide 5,20-Hemiacetal (8)——A solution of compound **7** (2.5 g) in chloroform (50 ml) was added dropwise to a solution of acetic anhydride (2.0 g) in chloroform (15 ml) in an ice bath. After being stirred for 2.5 h, the reaction mixture was washed with cold 5% aq. sodium bicarbonate. The solvent layer was dried over anhydrous sodium sulfate and concentrated to yield a brownish-yellow powder (1.8 g). The crude powder was chromatographed on silica gel (100 g) (eluent: benzene–methanol=500: 1 to 5: 1) to obtain a pale yellow powder (**8**, 150 mg). mp 103—105°C. UV λ_{max}^{MeOH} nm (ε): 281 (15770). MS *m/z*: 424 (M^+). ^{1}H-NMR δ (ppm): 0.97 (3H, t, J=6.0 Hz, C-17), 1.00 (3H, d, J=6.0 Hz, C-21), 1.23 (3H, d, J=6.0 Hz, C-18), 1.8 (3H, s, C-22), 2.86 (1H, m, C-8), 4.94 (1H, m, C-15), 5.5 (1H, t, C-20), 5.8 (1H, d, J=10.0 Hz, C-13), 6.3 (1H, d, J=15.0 Hz, C-10), 7.24 (1H, d, J=15.0 Hz, C-11). *Anal.* Calcd for $C_{23}H_{36}O_7$: C, 65.07; H, 8.55. Found: C, 65.22, H, 8.47.

Tylonolidedilactone (9)——Compound **8** (400 mg) dissolved in pyridine (0.5 ml) was added to a solution of CrO_3 (200 mg) in pyridine (2 ml). The whole was stirred at room temperature for 90 min, then water (2 ml) was added to the reaction mixture. The product was extracted with ethyl acetate (5 ml×3). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a pale yellow powder (**9**). The crude powder was purified by preparative TLC (silica gel, benzene–acetone=4: 1). mp 186—188°C. UV λ_{max}^{MeOH} nm (ε): 282 (16670). MS *m/z*: 422 (M^+, 422.505). IR (KBr): 1770 cm^{-1} (5-membered ring lactone ester), 1720 cm^{-1} (16-membered ring lactone ester), 1680 cm^{-1} (ketone carbonyl). ^{1}H-NMR δ (ppm): 3.57 (1H, d, $J_{2,3}$=11.4 Hz, C-3), 4.11 (1H, dd, $J_{4,5}$=10.8 Hz, $J_{5,6}$=4.2 Hz, C-5), 5.87 (1H, d, 10.5 Hz, C-13), 6.34 (1H, d, 15.5 Hz, C-10), 7.25 (1H, d, C-11).

Tylosin *N*-Oxide (11)——*m*-Chloroperbenzoic acid (3.0 g) was added to a solution of **1** (4.0 g) in chloroform (70 ml) under an argon atmosphere in an ice bath, and the solution was stirred for 4 h. The reaction mixture was washed with 10% aq. sodium sulfite. The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated off to yield compound **11** (3.5 g).

23-*O*-Mycinosyltylonolide 5,20-Hemiacetal (12)——A solution of acetic anhydride (2.5 g) in chloroform (20 ml) was added to a solution of crude **11** (3.5 g) in chloroform (50 ml) and the mixture was stirred in an ice bath for 3 h. When the reaction was completed, the mixture was washed with cold 5% aq. sodium bicarbonate. The solvent layer was dried over anhydrous sodium sulfate. After removal of the solvent, the resulting crude powder was purified by silica gel (100 g) column chromatography (eluent: benzene–methanol=500: 1 to 10: 1) to yield 200 mg of compound **12** as a white powder. UV λ_{max}^{MeOH} nm (ε): 282 (18700). MS *m/z*: 598 (M^+), 407 (aglycone), 191 (mycinose). ^{1}H-NMR δ (ppm): 0.95 (3H, t, J=7.0 Hz, C-17), 1.80 (3H, s, C-22), 3.55 (3H, s, C-2″ OCH_3), 3.62 (3H, s, C-3″ OCH_3), 4.55 (1H, d, J=10.0 Hz, C-13), 6.25 (1H, d, J=15.0 Hz, C-10), 7.35 (1H, d, J=15.0 Hz, C-11). *Anal.* Calcd for $C_{31}H_{50}O_{11}$: C, 62.19; H, 8.42. Found: C, 62.36; H, 8.64.

3,4″,4‴-Tri-*O*-acetyltylosin (14)——Tylosin (2 g) was dissolved in a mixture of acetic anhydride (2.7 ml) and pyridine (0.5 ml), and the mixture was stirred at room temperature for 20 h. The reaction mixture was poured into cold water, and the suspension was adjusted to pH 8.0 with sodium bicarbonate. The product was extracted with chloroform (100 ml×3) and the combined extract was washed with water. The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated off to obtain the acetate. The acetate was dissolved in methanol, and the solution was stirred at room temperature for 24 h. Removal of the solvent afforded the triacetate (**14**) as a pale yellow powder. MS *m/z*: 1041 (M^+), 449 (aglycone monoacetate), 232 (acetyl mycinose), 187 (acetyl mycarose), 174 (mycaminose). ^{1}H-NMR δ (ppm): 2.0—2.3 (9H, s, 3×$COCH_3$), 9.75 (1H, s, C-20).

3-Deoxy-3,6-bicyclotylosin (15)——A solution of compound **14** (50 mg) in ethanol (1.5 ml) was added to a solution of $LiOH \cdot H_2O$ (2.5 mg) in ethanol (1 ml), and the mixture was refluxed at 90°C for 1 h. After the concentration of the reaction mixture *in vacuo*, ethyl ether was added to the concentrate. The resulting precipitate was purified by preparative TLC (silica gel, benzene–acetone=1: 1) to give a white powder (**15**). mp 133—135°C. UV λ_{max}^{MeOH} nm (ε): 279 (18400). MS *m/z*: 897 (M^+), 391 (aglycone), 191 (mycinose), 174 (mycaminose), 145 (mycarose). ^{1}H-NMR δ (ppm): 9.75 (1H, d, J=3.5 Hz, C-20).

Demycarosylrelomycin (20)——A solution of compound **2** (30 g) in 50% methanol–0.2 M phosphate buffer (pH 7.5, 500 ml) was added to a suspension of $NaBH_4$ (0.8 g) in the same buffer, and the reaction mixture was stirred at room temperature for 19 h. The mixture was adjusted to pH 8.0 with 2 N aq. NaOH and was extracted with chloroform (500 ml×3). The organic layer was dried over anhydrous sodium sulfate

and the solvent was evaporated off *in vacuo* to yield an amorphous powder (**20**, 30 g). mp 95—96°C. $[\alpha]_D^{24}$ −16.2° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (36000). MS m/z: 773 (M^+), 599 (M^+−mycaminose), 598 (M^+−mycinose), 392 (aglycone), 175 (mycinose), 174 (mycaminose). ^{1}H-NMR δ (ppm): 1.77 (3H, s, C-22), 2.48 (6H, s, $N(CH_3)_2$), 3.44 (3H, s, C-2″ OCH_3), 3.56 (3H, s, C-3″ OCH_3), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.52 (1H, d, J=8.0 Hz, C-1″), 5.82 (1H, br d, $J_{13,14}$=11.0 Hz, C-13), 6.20 (1H, d, J=15.0 Hz, C-10), 7.22 (1H, d, J=15.0 Hz, C-11). ^{13}C-NMR (ppm): 60.5 (t, C-20), 33.5 (t, C-19).

20-Deoxy-20-iododemycarosylrelomycin (**21**)——A solution of iodine (19.7 g) in dry dimethylformamide (20 ml) was added dropwise to a solution of compound **20** (30 g) and triphenylphosphine (20.4 g) in dry dimethylformamide (50 ml). After being stirred for 2 h at room temperature under a nitrogen atmosphere, the reaction mixture was poured into cold aq. sodium bicarbonate (500 ml), and extracted with chloroform (100 ml×3). The organic layer was washed with 0.1 M aq. sodium thiosulfate (150 ml×2), then dried over anhydrous sodium sulfate, and the solvent evaporated off *in vacuo* to yield a brownish oil. The crude sample was purified by silica gel (1000 g) column chromatography (eluent: chloroform–methanol–conc. ammonia water=15: 1: 0.05) to give a white powder (**21**, 20.8 g). mp 102—104°C. $[\alpha]_D^{24}$ −46.6° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (13100). MS m/z: 883 (M^+), 756 (M^+−I), 692 (M^+−mycinose), 502 (aglycone), 375 (aglycone-I), 191 (mycinose), 190 (mycaminose). ^{1}H-NMR δ (ppm): 1.75 (3H, s, C-22), 2.48 (6H, s, $N(CH_3)_2$), 3.42 (3H, s, C-2″ OCH_3), 3.56 (3H, s, C-3″ OCH_3), 4.23 (1H, d, J=8.0 Hz, C-1′), 4.50 (1H, d, J=8.0 Hz, C-1″), 4.93 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.82 (1H, br d, $J_{13,14}$=11.0 Hz, C-13), 6.18 (1H, d, J=16.0 Hz, C-10), 7.26 (1H, d, J=16.0 Hz, C-11). ^{13}C-NMR δ (ppm): 41.7 (d, C-6), 33.2 (t, C-19), 5.2 (t, C-20).

20-Deoxydemycarosylrelomycin (**22**)——Compound **21** (6.0 g) and $NaBH_3CN$ (1.7 g) were dissolved in hexamethylphosphoric triamide (25 ml), and the suspension was stirred at 50°C for 24 h under a nitrogen atmosphere. The reaction mixture was worked up by dilution with cold water (250 ml), and extraction with chloroform (150 ml). The extract was washed with water, and the organic layer was dried over anhydrous sodium sulfate then evaporated to dryness under reduced pressure. The residue was subjected to silica gel column chromatography using a solvent system of chloroform–methanol–conc. ammonia water (10: 1: 0.05) to give a white powder (**22**, 4.27 g). mp 96—97°C. $[\alpha]_D^{24}$ −12.7° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (18300). MS m/z: 757 (M^+), 583 (M^+−mycaminose), 528 (M^+−mycinose), 392 (aglycone), 175 (mycinose), 174 (mycaminose). ^{1}H-NMR δ (ppm): 1.80 (3H, s, C-22), 2.56 (6H, s, $N(CH_3)_2$), 3.56 (3H, s, C-2″ OCH_3), 3.60 (3H, s, C-3″ OCH_3), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.54 (1H, d, J=8.0 Hz, C-1″), 4.95 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.80 (1H, br d, $J_{13,14}$=11.0 Hz, C-13), 6.18 (1H, d, J=16.0 Hz, C-10), 7.22 (1H, d, J=16.0 Hz, C-11). ^{13}C-NMR δ (ppm): 21.1 (t, C-19), 12.0 (q, C-20).

20-Deoxy-5-*O*-mycaminosylrelonolide (**23**)——A solution of compound **22** (2.5 g) in 0.5 N HCl (30 ml) was heated under reflux for 19 h. The reaction mixture was adjusted to pH 8.0 with 1 N aq. NaOH and was extracted with chloroform (100 ml×3). The organic layer was dried over anhydrous sodium sulfate, and removal of the solvent gave a crude powder. The crude powder was purified by silica gel column chromatography with chloroform–methanol–conc. ammonia water (15: 1: 0.05) to yield a colorless powder (**23**, 1.4 g). mp 110—112°C. $[\alpha]_D^{24}$ −6.2° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (19000). MS m/z: 583 (M^+, 583.373; Calcd for $C_{31}H_{53}NO_3$: 583.372), 393 (aglycone), 190 (mycaminose). ^{1}H-NMR δ (ppm): 1.82 (3H, s, C-22), 2.50 (6H, s, $N(CH_3)_2$), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.95 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.83 (1H, br d, $J_{13,14}$=10.0 Hz, C-13), 6.20 (1H, d, J=16.0 Hz, C-10), 7.22 (1H, d, J=16.0 Hz, C-11).

23-Deoxy-23-iodo-5-*O*-mycaminosyltylonolide (**24**)——A solution of iodine (2.6 g) in dimethylformamide (2 ml) was added dropwise to a solution of compound **3** (3.0 g) and triphenylphosphine (2.6 g) in dimethylformamide (5 ml). After being stirred for 25 h at room temperature under nitrogen atmosphere, the reaction mixture was worked up in a manner similar to that described above to obtain a crude paste. The crude sample was applied to a silica gel column using a solvent system of chloroform–methanol–conc. ammonia water (10: 1: 0.05) to give a white powder (**24**, 1.4 g). mp 116—117°C. $[\alpha]_D^{24}$ +46.6° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (18900). MS m/z: 707 (M^+), 580 (M^+−I), 517 (aglycone), 390 (aglycone-I), 190 (mycaminose). ^{1}H-NMR δ (ppm): 1.81 (3H, br s, C-22), 2.50 (6H, s, $N(CH_3)_2$), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.88 (1H, d t, $J_{14,15}$=4.0 Hz, $J_{15,16}$=8.0 Hz, C-15), 5.74 (1H, dd, $J_{13,14}$=12.0 Hz, $J_{13,22}$=1.0 Hz, C-13), 6.36 (1H, d, J=15.0 Hz, C-10), 7.40 (1H, d, J=15.0 Hz, C-11). ^{13}C-NMR δ (ppm): 45.7 (d, C-14), 5.2 (t, C-23).

Deepoxycirramycin A_1 (**19**)——Compound **24** (1.0 g) and $NaBH_3CN$ (350 mg) were suspended in hexamethylphosphoric triamide (10 ml), and the suspension was stirred under a nitrogen atmosphere at 50°C for 24 h. The reaction mixture was worked up in the usual way to afford a crude sample. The crude sample was chromatographed over silica gel using chloroform–methanol–conc. ammonia water (10: 1: 0.05) to obtain a white powder (**19**, 470 mg). mp 107—109°C. $[\alpha]_D^{24}$ −19.1° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (16000). MS m/z: 581 (M^+, 581.353; Calcd for $C_{31}H_{51}NO_9$: 581.356), 391 (aglycone), 190 (mycaminose). ^{1}H-NMR δ (ppm): 1.78 (3H, s, C-22), 2.47 (6H, s, $N(CH_3)_2$), 4.22 (1H, d, J=8.0 Hz, C-1′), 4.67 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.62 (1H, br d, $J_{13,14}$=9.0 Hz, C-13), 6.23 (1H, d, J=15.0 Hz, C-10), 7.22 (1H, d, J=15.0 Hz, C-11), 9.62 (1H, s, C-20). ^{13}C-NMR δ (ppm): 38.8 (d, C-14), 12.9 (q, C-23).

5-*O*-Mycaminosylrelonolide (**25**)——A solution of **3** (3.0 g) in 50% methanolic–0.2 M phosphate buffer (pH 7.5) (100 ml) was treated with $NaBH_4$ (75 mg) in the same buffer, and the suspension was stirred at room temperature for 19 h. The reaction mixture was adjusted to pH 8.0 with 2 N aq. NaOH, then extracted with chloroform (100 ml×3). The extract was dried over anhydrous sodium sulfate and evaporated to

dryness to afford a white powder (**25**, 3.0 g). mp 108—111°C. $[\alpha]_D^{24}$ −10.3° (c=1.0, MeOH), UV λ_{max}^{MeOH} nm (ε): 282 (20900). MS m/z: 599 (M^+), 409 (aglycone), 174 (mycaminose). ^{1}H-NMR δ (ppm): 1.83 (3H, s, C-22), 2.52 (6H, s, $N(CH_3)_2$), 4.33 (1H, d, J=7.0 Hz, C-1′), 4.99 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.95 (1H, br d, $J_{13,14}$=11.0 Hz, C-13), 6.28 (1H, d, J=16.0 Hz, C-10), 7.32 (1H, d, J=16.0 Hz, C-11).

20,23-Dideoxy-20,23-diiodo-5-*O*-mycaminosylrelonolide (26)——A solution of iodine (5.1 g) in dimethylformamide (5 ml) was added dropwise to a solution of compound **25** (3.0 g) and triphenylphosphine (5.3 g) in dimethylformamide (5 ml). After being stirred for 2 h at room temperature under a nitrogen atmosphere, the reaction mixture was treated in a manner similar to that described above to obtain a crude paste. The crude paste was applied to a silica gel column and eluted with chloroform–methanol–conc. ammonia water (10: 1: 0.05) to give a white powder (**26**, 2.1 g), mp 105—106°C. $[\alpha]_D^{24}$ +7.2° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (20900). MS m/z: 819 (M^+), 692 (M^+−I), 629 (aglycone), 565 (M^+−2I), 502 (aglycone-I), 375 (aglycone−2I), 174 (mycaminose). ^{1}H-NMR δ (ppm): 1.81 (3H, s, C-22), 2.52 (6H, s, $N(CH_3)_2$), 4.28 (1H, d, J=8.0 Hz, C-1′), 4.82 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.68 (1H, br d, $J_{13,14}$=10.0 Hz, C-13), 6.27 (1H, d, J=15.0 Hz, C-10), 7.49 (1H, d, J=15.0 Hz, C-11). ^{13}C-NMR δ (ppm): 45.5 (d, C-14), 32.9 (t, C-19), 9.3 (t, C-20), 5.3 (t, C-23).

5-*O*-Mycaminosylprotylonolide (27)——A suspension of compound **26** (2.0 g) and $NaBH_3CN$ (1.23 g) in hexamethylphosphoric triamide (5 ml) was stirred under a nitrogen atmosphere at 50°C for 8.5 h. The reaction mixture was worked up in the usual way to afford a crude paste. The crude sample was purified by silica gel column chromatography using a solvent system of chloroform–methanol–conc. ammonia water (15: 1: 0.05) to yield a white powder (**27**, 1.2 g). mp 95—96°C. $[\alpha]_D^{24}$ −14.1° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (23100). MS m/z: 567 (M^+, 567.378; Calcd for $C_{31}H_{53}NO_8$: 567.377), 377 (aglycone), 190 (mycaminose). ^{1}H-NMR δ (ppm): 1.78 (3H, s, C-22), 2.51 (6H, s, $N(CH_3)_2$), 4.27 (1H, d, J=7.0 Hz, C-1′), 4.68 (1H, d t, $J_{14,15}$=4.0 Hz, $J_{15,16}$=8.0 Hz, C-15), 5.57 (1H, br d, $J_{13,14}$=11.0 Hz, C-13), 6.19 (1H, d, J=16.0 Hz, C-10), 7.19 (1H, d, J=16.0 Hz, C-11). ^{13}C-NMR δ (ppm): 38.7 (d, C-14), 21.1 (t, C-19), 16.3 (q, C-20), 12.1 (q, C-23).

20-Deoxy-23-*O*-mycinosylrelonolide (28)——A solution of *m*-chloroperbenzoic acid (670 mg) in chloroform (10 ml) was added to a solution of compound **22** (2.5 g) in chloroform (20 ml). After being stirred for 3 h in an ice bath, the reaction mixture was washed with 10% aq. sodium sulfite (75 ml×2), and the organic layer was dried over anhydrous sodium sulfate. Removal of the solvent afforded the *N*-oxide (2.5 g). The *N*-oxide (2.0 g) and acetic anhydride (4.0 ml) were dissolved in chloroform (20 ml), and the mixture was heated under reflux for 2 h. The reaction mixture was poured into ice-sodium bicarbonate aq. (200 ml), and the whole was extracted with chloroform (100 ml×3). The organic layer was washed with aq. sodium bicarbonate (200 ml) and concentrated *in vacuo* to give a white powder. The crude powder was chromatographed on silica gel (75 g) (eluent: chloroform–methanol=50: 1) to obtain a white powder (**28**, 1.3 g). mp 81—82°C. $[\alpha]_D^{25}$ −27.6° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (22200). MS m/z: 584 (M^+), 566 (M^+-H_2O), 409 (aglycone). ^{1}H-NMR δ (ppm): 1.74 (3H, s, C-22), 3.36 (3H, s, C-2′ OCH_3), 3.48 (3H, s, C-3′ OCH_3), 4.42 (1H, d, J=8.0 Hz, C-1′), 4.82 (1H, b, C-15), 5.72 (1H, d, J=10.0 Hz, C-13), 6.08 (1H, d, J=15.0 Hz, C-10), 7.06 (1H, d, J=15.0 Hz, C-11).

23-Hydroxyprotylonolide (29)——Compound **28** (500 mg) was dissolved in 0.05 N HCl (100 ml), and the solution was heated under reflux for 23 h. After the reaction mixture had been neutralized, it was extracted with chloroform (100 ml×3). The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated off reduced pressure to obtain a crude powder. The crude powder was purified by silica gel (30 g) column chromatography (eluent: chloroform–methanol=30: 1) to give a white powder (**29**, 100 mg). mp 65—66°C. $[\alpha]_D^{25}$ −15.4° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (17800). MS m/z: 410 (M^+, 410.267; Calcd for $C_{23}H_{38}O_6$: 410.267), 392 (M^+-H_2O). ^{1}H-NMR δ (ppm): 1.78 (3H, s, C-22), 4.87 (1H, br t, $J_{15,16}$=8.0 Hz, C-15), 5.83 (1H, d, J=10.0 Hz, C-13), 6.21 (1H, d, J=16.0 Hz, C-10), 7.22 (1H, d, J=16.0 Hz, C-11).

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