Review Article

CHEMICAL AND BIOLOGICAL STUDIES ON 16-MEMBERED MACROLIDE ANTIBIOTICS

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New 16-membered macrolides were discovered one after another around 1967 to 1968 when structural studies on carbomycins and leucomycins came to a pause. With the exception of rosamicin (**40**) and chalcomycin (**16**), the majority of these antibiotics were discovered by Japanese researchers and this may be partly due to the fact that both leucomycin and spiramycin were being used clinically in Japan, in addition to the stimulation resulting from the structural determination of a series of leucomycin components. The discovery of a large number of components and their structural determination in a short period resulted from progress in the method of isolation of antibiotics, and the introduction of mass spectrometry in addition to NMR, IR, and UV spectrometry for structural determination.

Macrolide antibiotics show different biological and chemical properties such as antimycoplasma activity, induced resistance, structure of the lactone portion, mode of sugar binding and biosynthesis depending on the size of the lactone ring. The classfication of macrolides according to the size of the lactone ring was found to be quite rational.[1,2]

There are reviews on macrolide antibiotics in many papers[3-5] but there is none limited to 16-membered macrolides. We have, therefore, attempted to review recent studies on 16-membered macrolides, their interrelationship as revealed by structural determination of numerous components, their chemical and biological transformation, structure-activity relationships, and their biosynthesis.

1. Isolation and Structure of 16-Membered Macrolides

Following structural determination of old 16-membered macrolides such as carbomycin, leucomycin, chalcomycin (**16**) and spiramycin, many new components were discovered and their structures determined during the past 5 years. The total number of such macrolides has reached 40.

Table 1 summarizes the physicochemical properties of 16-membered macrolides which have been discovered to date and whose structures have been elucidated. These macrolides can be classified according their structure into two large groups, as shown in Fig. 1, depending on the difference in substituents at the 4-, 12-, 14-, and 15-positions of the lactone ring.

(A) Carbomycin-leucomycin group: This group of macrolides are differentiated by the difference of the structure in the 9~13 positions, of the acyl group in 3-position of the lactone ring, of the acyl groups in 4″-position of the mycarose moiety and their combinations. This group includes carbomycin, leucomycin, spiramycin, maridomycin, SF-837, YL-704, and espinomycin. The disaccharide, mycarosylmycaminose, is bonded to the 5-position of the lactone ring in all macrolides of this group.

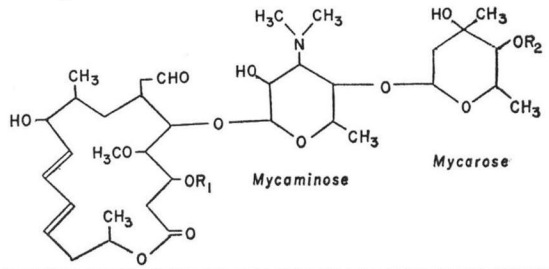
Table 1. Properties of 16-membered ring macrolide antibiotics

Antibiotic components	Producing organism	mp(°C)	Molecular formula	$[\alpha]_D$	UV λ_{max}	References
Carbomycin A (1)	*St. halstedii*	199.5~200.5	$C_{42}H_{67}O_{16}N$	−58.6°($CHCl_3$)		6,7
〃 B (2)	〃	141~144	$C_{42}H_{67}O_{15}N$	−35.0°(〃)		
Leucomycin A_1 (3)	*St. kitasatoensis*		$C_{40}H_{67}O_{14}N$	−66.0°(〃)	$E^{1\%}_{1cm}$ 232(400)	8~20
〃 A_3 (=Josamycin, YL-704 A_3) (4)	〃	120~121	$C_{42}H_{69}O_{15}N$	−55.4°(〃)	231.5(351)	
〃 A_4 (5)	〃	126~127	$C_{41}H_{67}O_{15}N$	−50.0°(〃)	231.5(375)	
〃 A_5 (6)	〃	120~123	$C_{39}H_{65}O_{14}N$	−52.0°(〃)	231.5(380)	
〃 A_6 (=YL-704 B_3) (7)	〃	135~137	$C_{40}H_{65}O_{15}N$	−56.0°(〃)	231.5(405)	
〃 A_7 (8)	〃		$C_{38}H_{63}O_{14}N$	−65.0°(〃)	232(405)	
〃 A_8 (9)	〃	147~149	$C_{39}H_{63}O_{15}N$	−58.3°(〃)	232(380)	
〃 A_9 (10)	〃		$C_{37}H_{61}O_{14}N$	−65.1°(〃)	232(395)	
〃 U (11)	〃					
〃 V (12)	〃					
Spiramycin I (13)	*St. ambofaciens*	133~137	$C_{43}H_{74}O_{14}N_2$	−96.0°(MeOH)	232(322)	21~24
〃 II (14)	〃	130~133	$C_{45}H_{76}O_{15}N_2$	−80.0°(〃)	232(307)	
〃 III (15)	〃	128~131	$C_{46}H_{78}O_{15}N_2$	−85.0°(〃)	232(327)	
Chalcomycin (16)	*St. bikiniensis*	121~123	$C_{35}H_{56}O_{14}$			25~27
Neutramycin (17)	*St. rimosus*	222~223	$C_{34}H_{54}O_{14}$	−34.5°(EtOH)	216(340)	28,29
Tylosin (18)	*St. fradiae*	128~132	$C_{45}H_{77}O_{17}N$	−46.0°(MeOH)	282(245)	30,31
Relomycin (19)						32
Angolamycin (=Sincomycin A) (20)	*St. eurythermus*	134~136	$C_{46}H_{77}O_{17}N$	−64.0°($CHCl_3$)	log ε 240(4.16)	33~35
Niddamycin (21)	*St. djakartensis*	132~136	$C_{40}H_{65}O_{14}N$	−43.0°(MeOH)	$E^{1\%}_{1cm}$ 279(275)	36
Cirramycin A_1 (22)	*St. cirratus*	124~128	$C_{31}H_{51}O_{10}N$			37~41
B-58941 (23)	*St. fradiae*	229	$C_{37}H_{59}O_{12}N$	−88.4°($CHCl_3$)	log ε 240(4.21)	42,43
SF-837 A_1 (=YL-704 B_1, Espinomycin A_1) (24)	*St. mycarofaciens*	122~124	$C_{41}H_{67}O_{15}N$	−67.0°(EtOH)	223 (ε 26,000)	44~48
〃 A_2 (25)	〃	125~128	$C_{42}H_{69}O_{15}N$	−68.0°(〃)	$E^{1\%}_{1cm}$ 232(320)	
〃 A_3 (26)	〃	122~125	$C_{41}H_{65}O_{15}N$	−44.0°(〃)	280(295)	
〃 A_4 (27)	〃	120~122	$C_{42}H_{67}O_{15}N$	−40.0°(〃)	280(285)	
YL-704 A_0 (28)	〃		$C_{44}H_{72}O_{15}N$		log ε	49~53
〃 A_1 (29)	〃	122~123	$C_{43}H_{71}O_{15}N$	−50.2°($CHCl_3$)	232.5(4.45)	
〃 A_3 (=Leucomyin A_3) (4)	〃	121~122	$C_{42}H_{69}O_{15}N$	−54.0°(〃)	232(4.45)	
〃 B_1 (=SF-837 A_1, Espinomycin A_1) (24)	〃	131~132	$C_{41}H_{67}O_{15}N$	−42.1°(〃)	232.5(4.36)	
〃 B_3 (7) (=Leucomycin A_6)	〃	136~137	$C_{40}H_{65}O_{15}N$	−55.0°(〃)	232(4.40)	
〃 C_1 (30) (=Maridomycin III)	〃	125~127	$C_{41}H_{67}O_{16}N$	−69.0°(〃)	end	
〃 C_2 (31) (=Espinomycin A_3, Maridomycin II)	〃	116~118	$C_{40}H_{65}O_{15}N$	−42.0°(〃)	232.5(4.43)	
〃 C_3 (32) (=Maridomycin I)	〃	126~127	$C_{43}H_{71}O_{16}N$		end	
〃 C_4 (33)	〃	130~132	$C_{42}H_{69}O_{16}N$		end	
〃 W_1 (34)	〃	159~161	$C_{43}H_{69}O_{15}N$	−32.0°(〃)	280(4.37)	

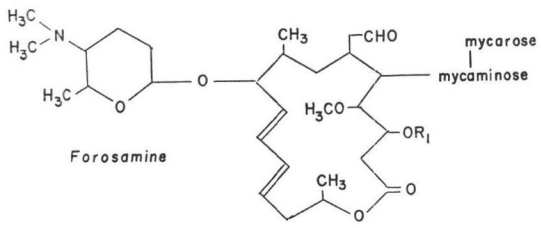
Table 1.

Antibiotic components	Producing organism	mp(°C)	Molecular formula	$[\alpha]_D$	UV λ_{max}	References
〃 W_2 (35)	〃	101~103	$C_{44}H_{71}O_{15}N$		280(4.37)	
Maridomycin I (=YL-704 C_3) (32)	*St. hygroscopicus*	129~132	$C_{43}H_{71}O_{16}N$	−72.3°(EtOH)	end	54~57
〃 II (=YL-704 C_2, Espinomycin A_3) (31)	〃	134~136	$C_{42}H_{69}O_{16}N$	−71.9°(〃)	〃	
〃 III (YL-704 C_1) (30)	〃	135~138	$C_{41}H_{67}O_{16}N$	−76.0°(〃)	〃	54,57
〃 IV (36)	〃	143~146	$C_{40}H_{67}O_{16}N$	−76.2°(〃)	〃	
〃 V (37)	〃	143~146	$C_{40}H_{65}O_{16}N$	−73.2°(〃)	〃	
〃 VI (38)	〃	149~154	$C_{39}H_{63}O_{16}N$	−77.7°(〃)	〃	
Espinomycin A_1 (=SF-837 A_1) (24)	*St. furdicidicus*	159	$C_{41}H_{67}O_{15}N$	−69.0°(〃)	231.5	58,59
〃 A_2 (39)	〃	143	$C_{42}H_{69}O_{15}N$	−64.0°(〃)	231.5	
〃 A_3 (YL-704 C_2, Maridomycin II) (31)	〃					
Rosamicin (40)	*Micromonospora rosaria*	119~122	$C_{31}H_{51}O_9N$	−35.0°(〃)	240 (ε 14,600)	60,61

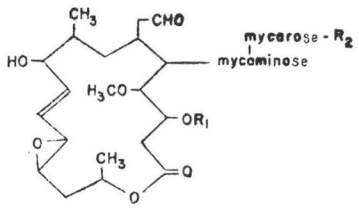
Fig. 1. Structures of 16-membered macrolides



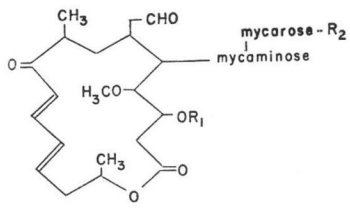
			No.	R_1	R_2
Leucomycin	A_1		3	H	$COCH_2CH(CH_3)_2$
	A_3	(Josamycin) (YL-704 A_3)	4	$COCH_3$	$COCH_2CH(CH_3)_2$
	A_4		5	$COCH_3$	$COCH_2CH_2CH_3$
	A_5		6	H	$COCH_2CH_2CH_3$
	A_6	(YL-704 B_3)	7	$COCH_3$	$COCH_2CH_3$
	A_7		8	H	$COCH_2CH_3$
	A_8		9	$COCH_3$	$COCH_3$
	A_9		10	H	$COCH_3$
	U		11	$COCH_3$	H
	V		12	H	H
LY-704	A_1		29	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
SF-837	A_2		25	$COCH_2CH_3$	$COCH_2CH_2CH_3$
Espinomycin	A_2		39	$COCH_2CH_3$	$COCH(CH_3)_2$
SF-837	A_1	(YL-704 B_1) (Espinomycin A_1)	24	$COCH_2CH_3$	$COCH_2CH_3$
YL-704	C_2	(Espinomycin A_3) (Maridomycin II)	31	$COCH_2CH_3$	$COCH_3$
YL-704	A_0		28	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$

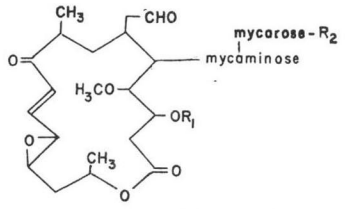


		R_1
Spiramycin I	13	H
II	14	$COCH_3$
III	15	$COCH_2CH_3$



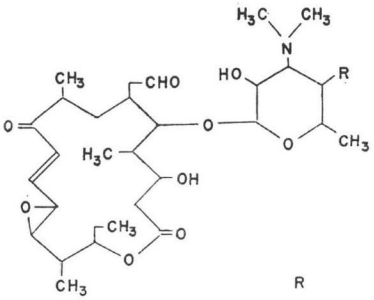
			R_1	R_2
Maridomycin	I	(YL-704 C_3)	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
	II	(YL-704 C_2)	$COCH_3$	$COCH_2CH(CH_3)_2$
	III	(YL-704 C_1)	$COCH_2CH_3$	$COCH_2CH_3$
	IV		$COCH_3$	$COCH_2CH_3$
	V		$COCH_2CH_3$	$COCH_3$
	VI		$COCH_3$	$COCH_3$

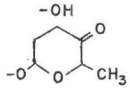


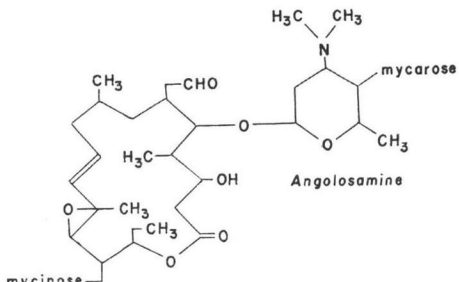


Carbomycin A I R_1 = $COCH_3$, R_2 = $COCH_2CH(CH_3)_2$

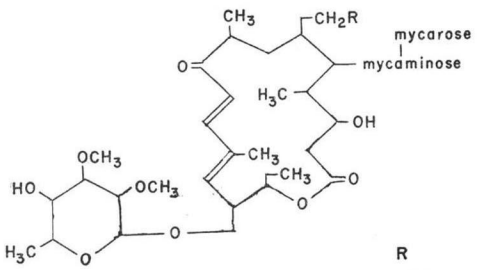
			R_1	R_2
Carbomycin	B	2	$COCH_3$	$COCH_2CH(CH_3)_2$
YL-704	W_1	34	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
SF-837	A_4	27	$COCH_2CH_3$	$COCH_2CH_2CH_3$
SF-837	A_3	26	$COCH_2CH_3$	$COCH_2CH_3$
YL-704	W_2	35	$COCH_2CH_2CH_3$	$COCH_2CH(CH_3)_2$



		R
Cirramycin A_1	22	-OH
B-58941	23	
Rosamicin	40	-H

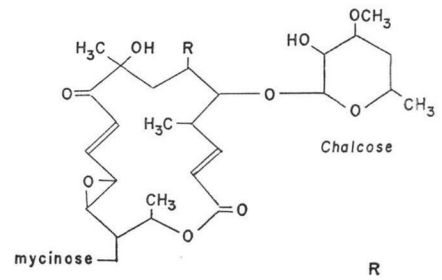


20
Angolamycin (= Sincomycin A)



Mycinose

	R
Tylosin 18	CHO
Relomycin 19	CH_2OH



	R
Chalcomycin 16	CH_3
Neutramycin 17	H

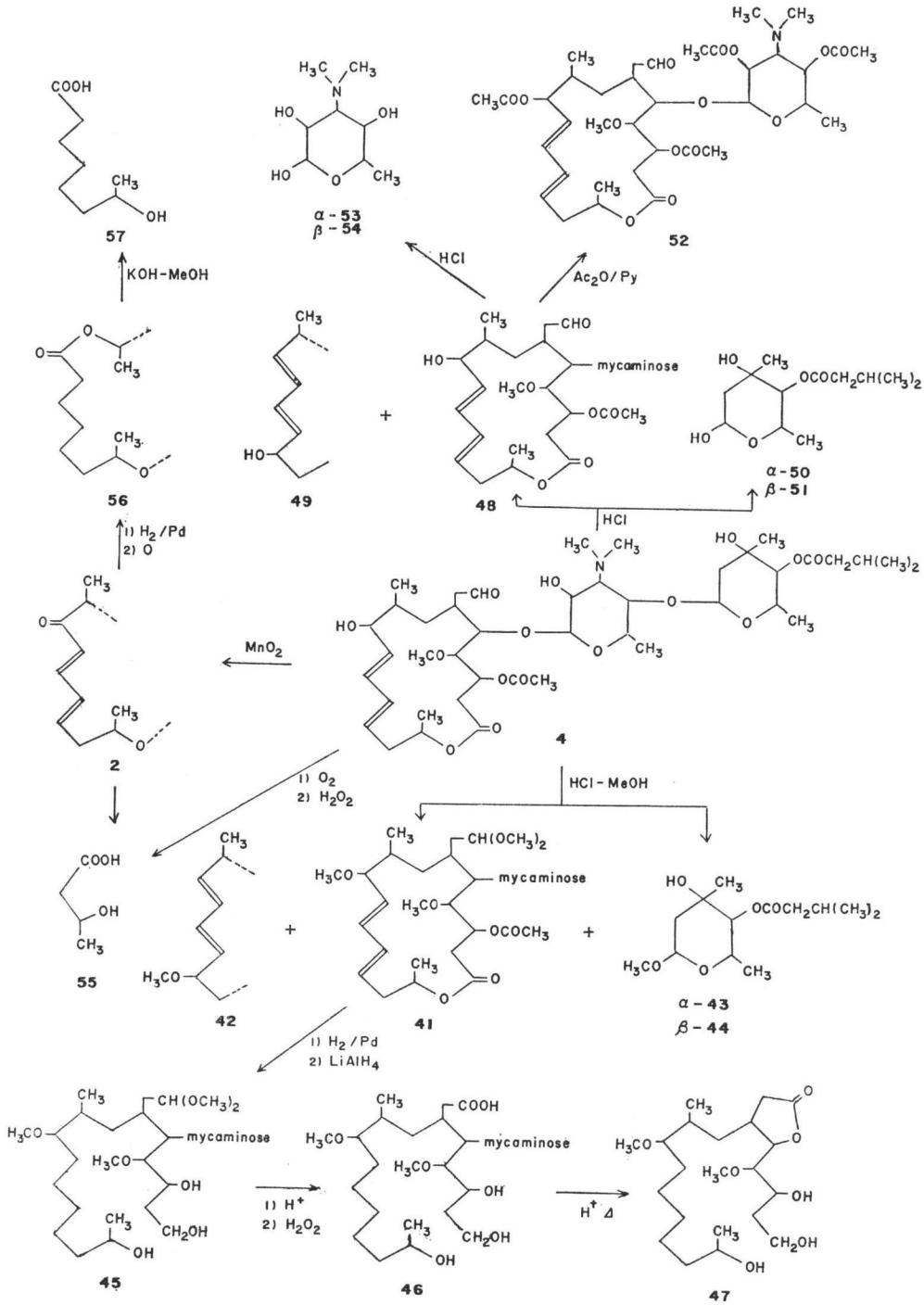
(B) Tylosin-chalcomycin group: The macrolides of this group have, in common, a methyl group in the 4- and 12-positions, mycinose through the methyl or methylene group at the 14-position and an ethyl group in the 15-position of the lactone ring. Tylosin (**18**), cirramycin A_1 (**22**), rosamicin (**40**), angolamycin (**20**) and B-58941 (**23**) are the antibiotics of this group. Rosamicin (**40**) is produced not by a *Streptomyces* but by a *Micromonospora*, and this is the only 16-membered macrolide that has the amino sugar, desosamine, bonded to the 5-position of the lactone ring instead of mycaminose, as occurs in 14-membered macrolides such as erythromycin and oleandomycin. Angolamycin (**20**) has a structure similar to tylosin (**18**) and differs from other macrolides in angolosamine lacking a 2′-OH instead of mycaminose bonded to the 5-position. B-58941 (**23**) is a compound with a neutral sugar *i.e.*, 2, 3, 4-trideoxy-L-hexopyranosyl-4-ulose in the 4′-position of mycaminose instead of mycarose and this sugar is of interest biosynthetically because of its relation to mycarose. Relomycin (**19**) has the same structure as tylosin (**18**) but with its CHO group reduced to CH_2OH. In the case of the neutral macrolides, chalcomycin (**16**) and neutramycin (**17**), hydroxyl or acyl groups in the 3-position and formyl methyl group in the 6-position are lacking and the latter is replaced with tertiary OH and methyl groups. They are characterized by having a neutral sugar, chalcose, in place of mycaminose.

In this group of macrolides, many studies were made and new components discovered during the 1970s in antibiotics related to the leucomycin group. After structural studies on carbomycins and leucomycins has been completed to some extent, related macrolides, such as SF-837, maridomycin, and YL-704, were discovered and their structure determined in a short period of time.

Fundamental reactions in the structural determination of leucomycins are illustrated in Chart 1. Treatment of leucomycin A_3 (**4**) with dilute hydrochloric acid gives α, β-isovaleryl-mycarose (**50, 51**), and drastic acid hydrolysis of demycarosyl leucomycin A_3 (**48**) gives α, β-mycaminose (**53, 54**). These neutral and basic sugars were found to have α- and β-configurations in leucomycin, respectively, as determined from their NMR and IR spectral data. In the lactone portion, the nature of the skeleton at the C-13 to C-15 segment and the presence of a methyl group at the C-15 position revealed by the formation of β-hydroxybutyric acid (**55**) by ozone and hydrogen peroxide oxidation of leucomycin A_3 (**4**) and 9-dehydro leucomycin A_3 (**2**). UV spectral data of absorption at 232 nm suggested the presence of an α, β, γ, δ-unsaturated alcohol and its shift to 280 nm by manganese dioxide oxidation suggested the presence of an α, β, γ, δ-unsaturated ketone. The diene was found to have a *trans-trans* configuration from

the NMR spectrum. The position of the CHO group in the lactone portion was determined by making its acetal derivative (**41**), its catalytic reduction with palladium carbon to the tetrahydro compound, its transformation to the octahydro compound (**45**) by reduction with

Chart 1. Structure determination of leucomycin A_3 (**4**)

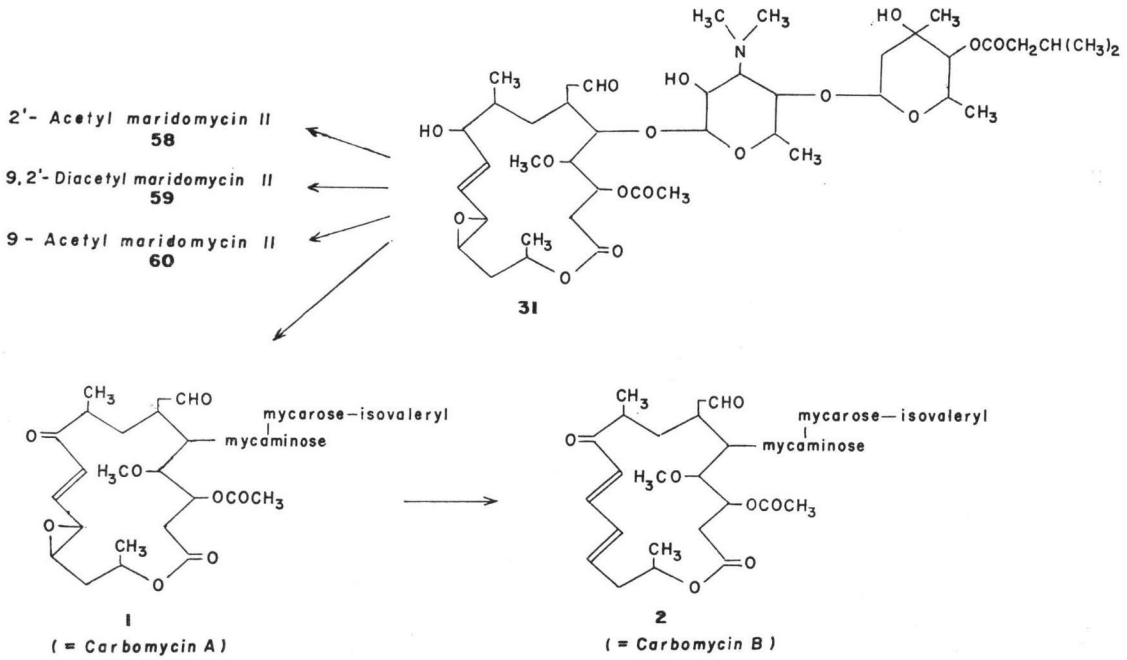


lithium aluminum hydride, the liberation of its CHO group with dilute acid, oxidation to COOH with hydrogen peroxide, thereby generating the amphoteric substance (**46**). Removal of mycaminose from this substance by heating with hydrochloric acid gave a substance (**47**) which showed an IR absorption at 1770 cm^{-1} for a 5-membered lactone. This fact indicates that mycaminose was bonded to the position γ to CHO group. 9-Dehydro leucomycin A_3 (**2**) was found to be identical with the structurally established carbomycin B (**2**). As for the absolute structure of leucomycin A_3 (**4**), X-ray cystallographic analysis was made on the hydrobromide of demycarosyl-isoleucomycin A_3 (**49**), in which the OH group at 9-position had undergone allyl rearrangement of the 13-position and the configuration of the parts other than the allyl rearrangement portion was determined. The steric configuration of the methoxyl group in the 4-position, presumed earlier for carbomycin B, had to be corrected.

The proof of the position and absolute structure of the OH group in the 9-position remained unresolved but the position of the OH group was proved from NMR spectral data and formation of 7-hydroxyoctanoic acid (**57**) by the BAYER-VILLIGER oxidation of 9-dehydrotetrahydro compound and its subsequent hydrolysis. The hydroxyl in the 9-position was selectively converted to the 2, 4-dinitrobenzoate and following application of MILL's Rule and the benzoate rule, the absolute configuration at C-9 was assingned the *R*-specification. Recently, however, a report[62] appeared which described comparative NMR and IR spectral examinations on 9-epi-leucomycin A_3 and natural leucomycin A_3, which were isolated by the sodium borohydride reduction of the dimethylacetal of niddamycin (**21**) and the absolute configuration at C-9, which bears the OH group, was found to have the *S*-specification.

The main reactions of maridomycin, YL-704, and SF-837, the macrolides related to the

Chart 2. Structure determination of maridomycin II (**31**)



carbomycin-leucomycin group are given in Charts 2, 3, and 4, respectively. The structure of these macrolides was revealed by their structural correlation to carbomycin and leucomycin.

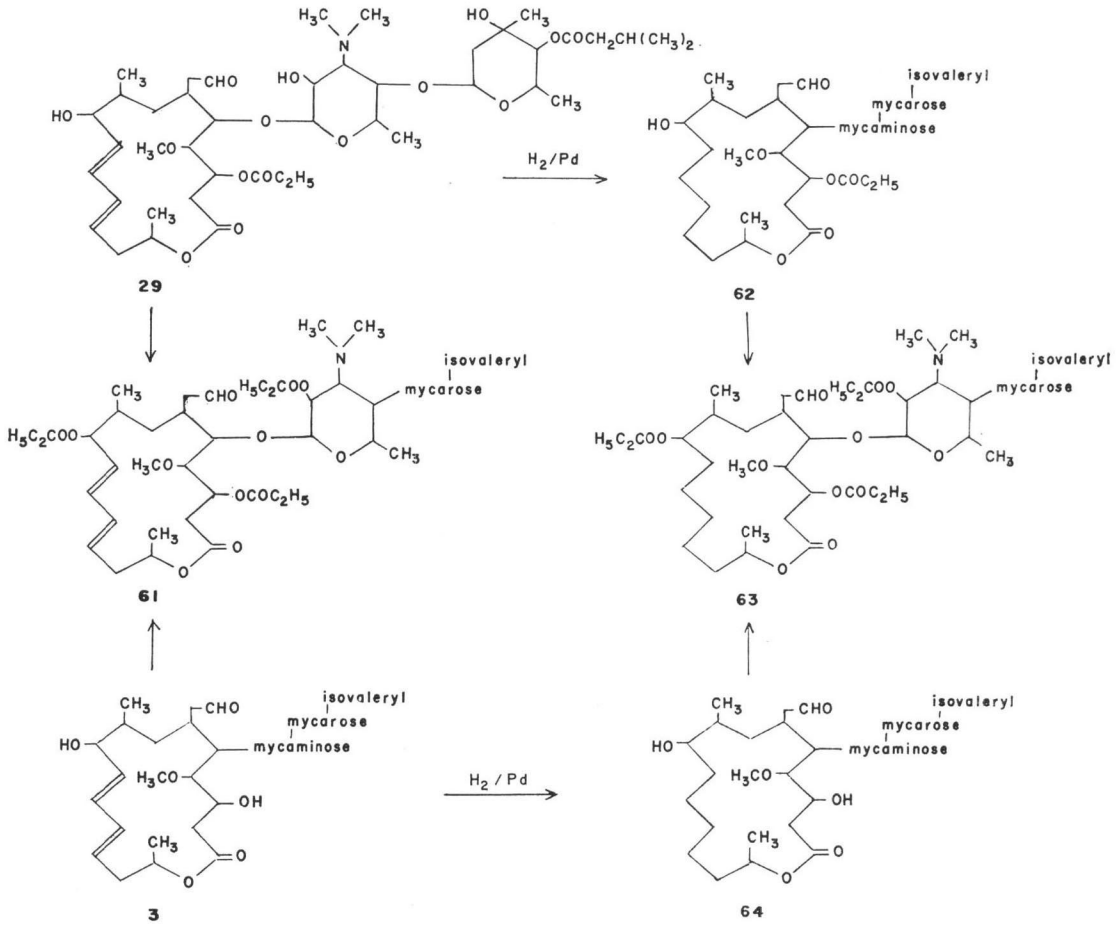
Maridomycin possesses an epoxide in the 12~13 position. Oxidation of the 9-OH with chromium trioxide-pyridine and severance of the epoxide with potassium iodide in acetic acid gave 9-dehydro-deepoxy-maridomycin II (**2**) which was found to be identical with carbomycin B (**2**).

The structure of YL-704 A_1 (**29**), was determined by identification of 9, 2′-dipropionyl-YL-704 A_1 (**61**) with the 3, 9, 2′-tripropionate of leucomycin A_1 (**3**), and that of the propionylated compounds (**63**) obtained from the tetrahydro compounds (**62**, **64**) of YL-704 A_1 (**29**) and leucomycin A_1 (**3**).

9, 2′-Propionyl SF-837 A_1 (**65**) was found to be identical with 3, 9, 2′-4″-tetrapropionate (**65**) of leucomycin V (**12**). The demycarosyl compound (**67**) of SF-837 A_1 (**24**), obtained by acid hydrolysis of tetrahydro-SF-837 A_1 (**66**), was found to be identical with tetrahydro-forocidine III (**67**), obtained by acid hydrolysis of tetrahydro-spiramycin III (**68**).

In parallel with the revelation of structural correlations of the 16-membered macrolides,

Chart 3. Structure determination of YL-704 A_1 (**29**)



structural analysis of these macrolides by mass spectrometry was carried out, especially on minute amounts of metabolites and chemical reaction products. SUZUKI[63] applied high-resolution mass spectrometry to the acetylated compounds of each component of YL-704 and found that the main peaks can be classified into five groups, each group reflecting the structural characteristics as in next page.

Chart 4. Chemical correlation of antibiotic SF-837 with leucomycin and spiramycin

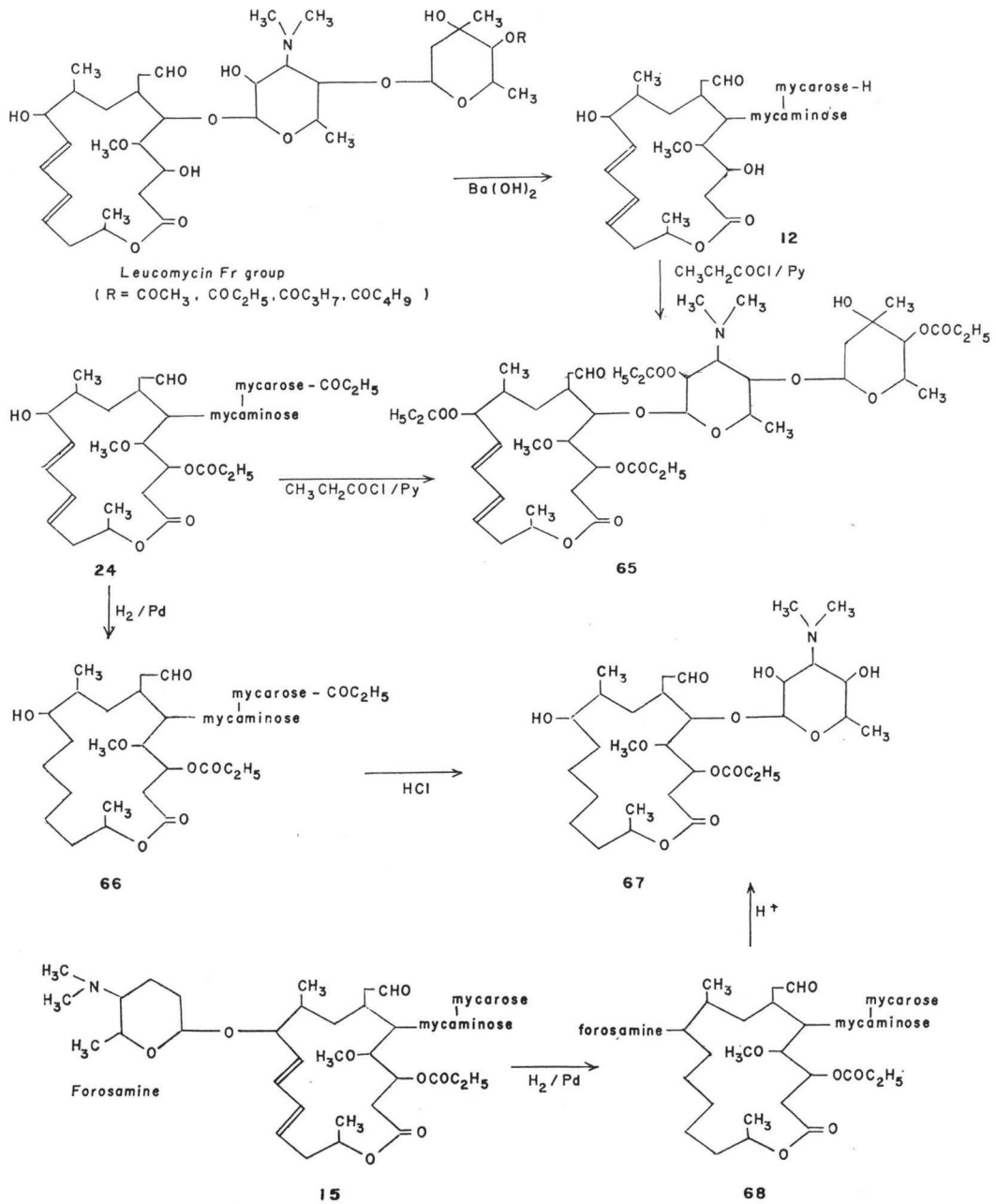


Fig. 2. Mass spectrum of diacetyl YL-704 A_1 (**69**)

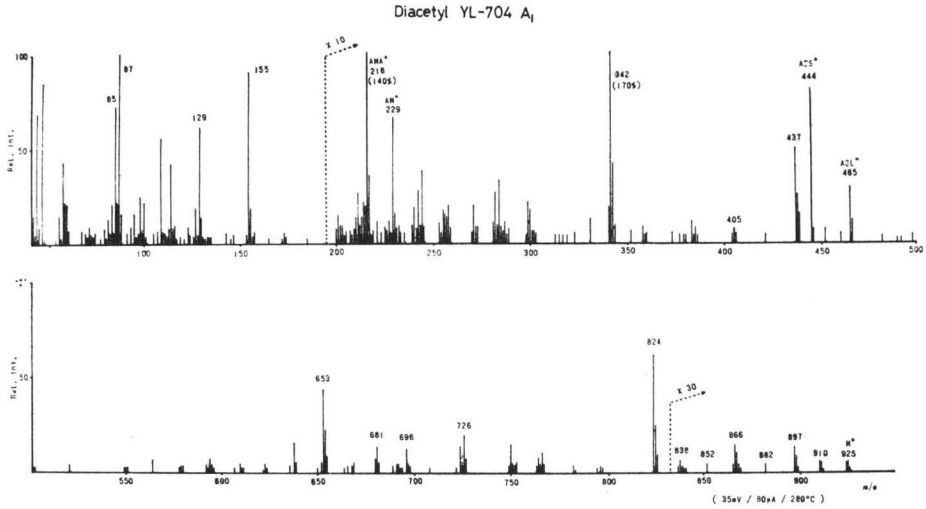
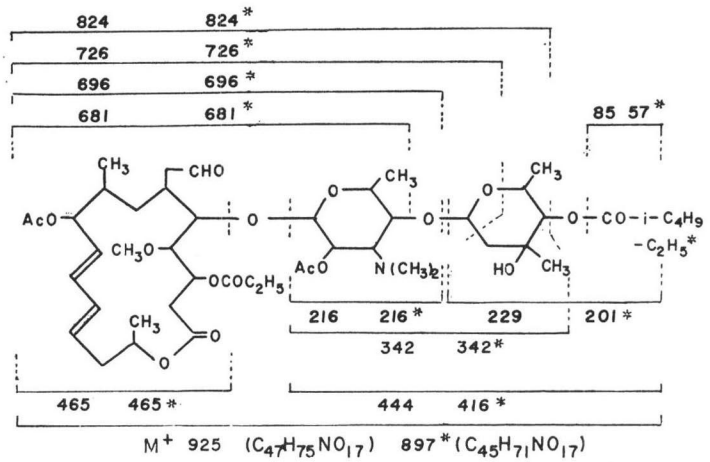


Fig. 3. Diagnostic fragmentations of diacetyl YL-704 A_1 (**69**) and diacetyl YL-704 B_1* (**7**)



Group 1: *m/e* 925 (M^+, 925.5048. $C_{47}H_{75}O_{17}N$, 925.5035)
m/e 910 - 824, molecular ion(s) and its (their) fragment(s)
Group 2: *m/e* 726 - 653, aglycone-sugar bonding ion
Group 3: *m/e* 465 - 405, aglycone (AGL^+) and acyl -disaccharide (ADS^+) ions
Group 4: *m/e* 342 - 129, ions related to sugars
Group 5: *m/e* 85, terminal acyl ions

The structural characteristics of the aglycone moiety appear in Group 3, those of disaccharides in Group 4, and the fragments synthesized in Groups 3 and 4 appear in Group 2.

Fig. 2 shows the mass spectrum of the diacetyl YL-704 A_1 (**69**), indicated by SUZUKI and others. Fig. 3 shows the diagnostic fragmentation diagrams; that of diacetyl YL-704 A_1 (**69**) in which the 4-position of mycarose has an isovaleryl group and of diacetyl YL-704 B_1 (**70**) in which the same position has a propionyl group. The information obtained was also confirmed

Fig. 4. Diagnostic fragmentation of maridomycin II (31)

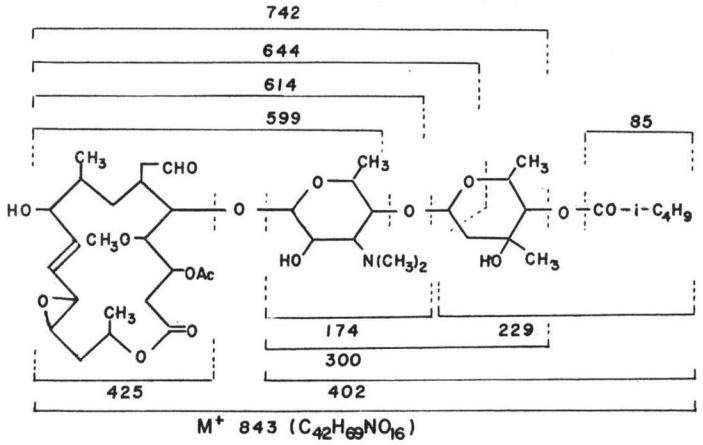
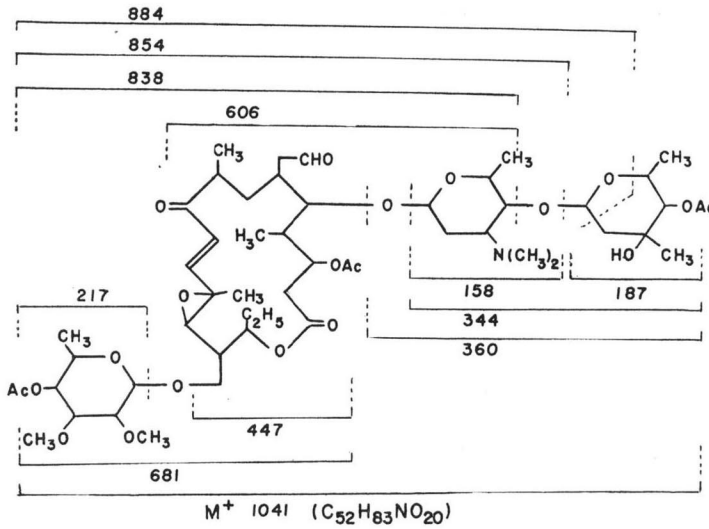


Fig. 5. Diagnostic fragmentation of triacetyl angolamycin (71) (=Triacetyl shincomycin A)



by the shift method in deuterio-diacetylated derivatives of YL-704. The use of this technique made it possible to carry out identification and structural analysis of the aglycone and sugar moieties rationally according to changes in their functional and substituent groups.

Figs. 4 and 5 also show the diagnostic fragmentation diagram of maridomycin II (**31**), which has an epoxide at the 12~13 position on the lactone ring, and the triacetate (**71**) of angolamycin (**20**) structurally related to tylosin (**18**). It was found by comparison of mass spectra that shincomycin A (**20**), reported in 1965, was identical with angolamycin (**20**), though the position of the binding of disaccharide to the aglycone in these macrolide was established by ^{13}C-NMR spectrometry.[64]

PAUL and TCHELITCHEFF[21,22] proposed an 18-membered cyclic structure for spiramycin in 1965 but this was corrected to a 17-membered ring by KUEHNE and BENSON[23] in 1965. Later, we[24] further corrected this to a 16-membered ring structure from biosynthetic evidence and

Fig. 6. ^{13}C-NMR spectrum of leucomycin A_3 (**4**) ($CDCl_3$, 22.63 MHz)

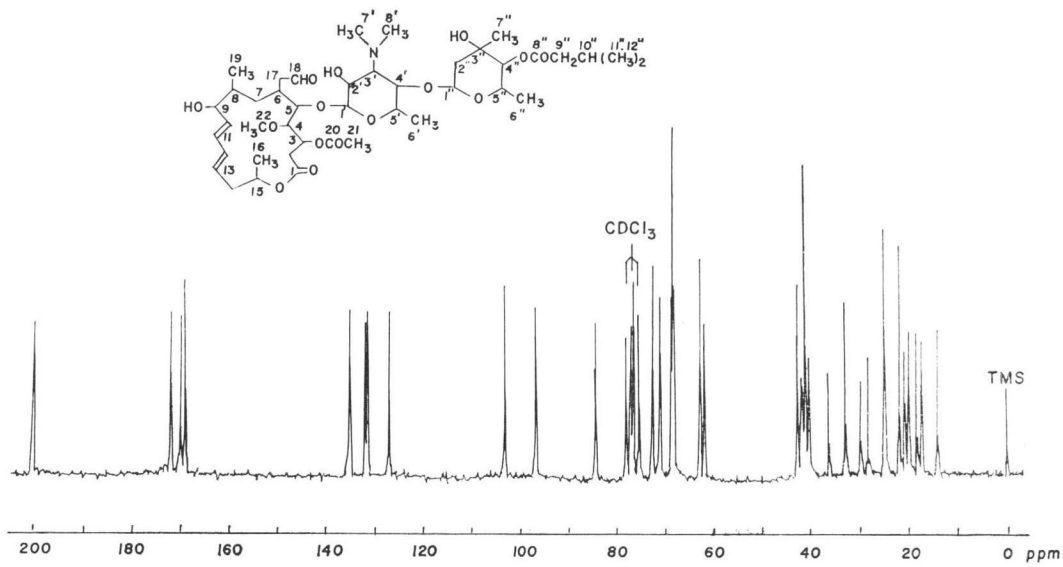


Table 2. Chemical shifts of leucomycin A_3 (**4**)

Aglycone				Mycaminose		Mycarose	
C—1	169.9 ppm	C—13	132.1 ppm	C—1′	103.7 ppm	C—1″	97.0 ppm
C—2	37.0	C—14	40.9$_c$	C—2′	69.0$_d$	C—2″	41.9
C—3	71.9	C—15	68.8$_d$	C—3′	69.0$_d$	C—3″	69.3
C—4	77.5	C—16	20.3	C—4′	76.0	C—4″	77.1
C—5	84.9	C—17	42.4$_c$	C—5′	72.9	C—5″	63.5
C—6	28.8$_a$	C—18	201.2	C—6′	18.8$_e$	C—6″	17.8$_e$
C—7	30.4$_a$	C—19	14.7	C—7′	41.9	C—7″	25.5
C—8	33.5	C—20	170.8	C—8′	41.9	C—8″	172.9
C—9	73.1	C—21	21.3			C—9″	43.3
C—10	135.7$_b$	C—22	62.4			C—10″	25.5
C—11	127.6$_b$					C—11″	22.4
C—12	132.6$_b$					C—12″	22.4

a, b, c, d, e: assignments within any vertical column may be reversed.

the behavior in the NMR spectrum of the aldehyde group in spiramycin and proposed that it had the same absolute structure as leucomycins.

With respect to the structure of 16-membered ring macrolides, the configuration of the glycoside bonding of forosamine linked to the 9-position in spiramycin had remained undetermined as well as also whether the bonding position of mycaminose to the aglycone in tylosin (**18**)[30] was 3 or 5. We carried out stereochemical analyses of 16-membered macrolides by ^{13}C-NMR spectrometry[64] and these unsolved points were elucidated. In order to assign each of the carbon atoms in the ^{13}C-NMR spectra of 16-membered ring macrolides, leucomycins and their derivatives, carbomycins, spiramycins, tylosin (**18**), cirramycin A_1 (**22**), chalcomycin (**16**), and neutramycin (**17**), the sugars bonded to their aglycone portion, mycaminose, mycarose, mycinose, forosamine, and chalcose, were either isolated from these macrolide antibiotics or

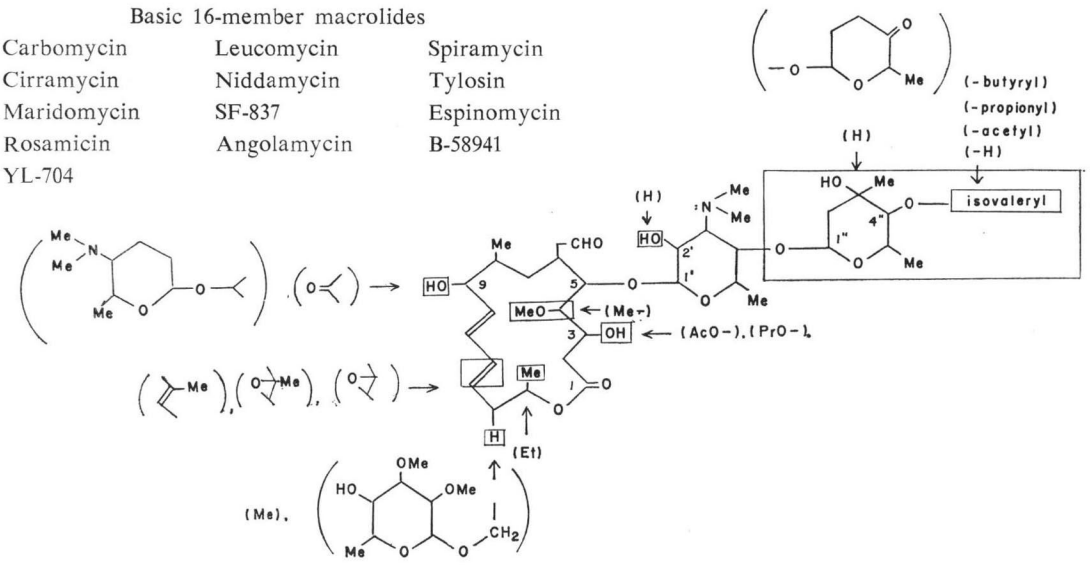
newly synthesized and their methyl glycosides were analyzed. Then each of the carbon atoms in the lactone portion was assigned by structural comparison of each of these macrolides. Fig. 6 shows the ^{13}C-NMR spectrum of leucomycin A_3 (**4**) in $CDCl_3$, and the values of chemical shift of the assigned carbon atoms are given in Table 2.

As will be clear from these spectral charts and data, the signal in the lowest magnetic field at δ 201.2 is due to CHO; that of the estercarbonyl of the lactone ester, acetyl in the 3-position, and isovaleryl in the 4-position of mycarose appear at around δ 170 and four olefin carbons of C-10 to C-13 at δ 127~136. The signals at δ 103.7 and 97.0 were assigned to the β-anomeric carbon of mycaminose and α-anomeric carbon of mycarose, respectively. From these results, forosamine was found to have a β-configuration, since spiramycin shows a signal corresponding to the β-anomeric carbon at δ 100.3. This fact was also confirmed by the measurement of $J_{^{13}C-^{1}H}$ of α, β-anomeric carbon. Further, the chemical shift of the carbonyl-carbon in the lactone ester of tylosin (**18**) revealed a shift to a lower magnetic field due to the intramolecular hydrogen bonding of the 3-OH with the ester carbonyl and mycaminose was found to be bonded to the 5-position to the lactone ring.[64]

Chart 5. Structural relationship of basic 16-membered macrolides

Basic 16-member macrolides

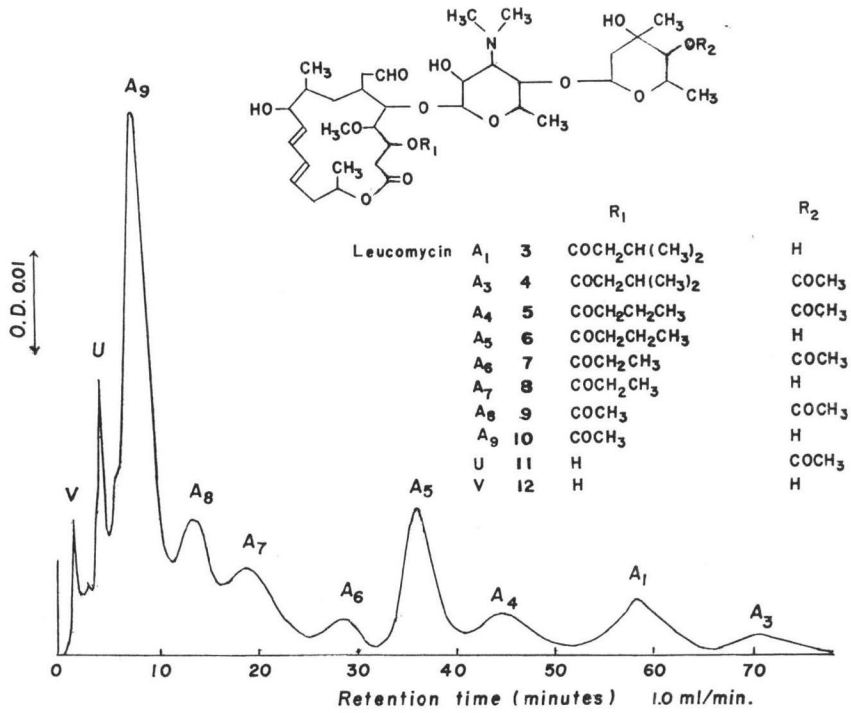
Carbomycin	Leucomycin	Spiramycin
Cirramycin	Niddamycin	Tylosin
Maridomycin	SF-837	Espinomycin
Rosamicin	Angolamycin	B-58941
YL-704		



This seems to have brought structural studies of 16-membered macrolides to the end of the first stage. Correlation among the structure of basic 16-membered macrolides, excluding the neutral macrolides, chalcomycin (**16**) and neutramycin (**17**), is summarized in Chart 5, centered around leucomycin A_1 (**3**), one of the components of leucomycin. There is a good possibility that new related macrolides may be discovered in the future, especially by the difference of combinations of the acyl group in the 3-position of the lactone ring and the acyl group in 4″-position of mycarose, and the structure in the C-9 to C-13 positions, as shown surrounded by a rectangle in Chart 5. Separation and detection of macrolides with extremely similar structure and with a large molecular weight are highly difficult. Fast liquid chromatography has been applied to the detection of each of the components of these macrolides, and separa-

tion and detection of each of the components A_1 (**3**) to A_8 (**10**) of leucomycin by this technique in a short time and in high efficiency have been reported[65] (Fig. 7). This fact indicates that this technique offers a powerful means for the discover of new macrolides, *i.e.*, for their separation for the purpose of identification.

Fig. 7. Fast liquid chromatogram of leucomycin complex
Instrument: JASCO FLC-150, Detector: JASCOUVIDEC-2(232.5 nm) Spectrophtometer. Separation mode: Partition. Column: JASCOPACK SV-02-100. Column temp.: Ambient. Column Pressure: 100 kg/cm². Mobile phase: M/15 Acetate Buffer (pH 4.9)-MeOH-acetonitrile (60 : 32 : 3)



Leucomycin			R_1	R_2
	A_1	**3**	$COCH_2CH(CH_3)_2$	H
	A_3	**4**	$COCH_2CH(CH_3)_2$	$COCH_3$
	A_4	**5**	$COCH_2CH_2CH_3$	$COCH_3$
	A_5	**6**	$COCH_2CH_2CH_3$	H
	A_6	**7**	$COCH_2CH_3$	$COCH_3$
	A_7	**8**	$COCH_2CH_3$	H
	A_8	**9**	$COCH_3$	$COCH_3$
	A_9	**10**	$COCH_3$	H
	U	**11**	H	$COCH_3$
	V	**12**	H	H

Conformational analysis of macrolide antibiotics in solution will be an important factor in examining their structure-activity correlation and chemical reactivity. There have been many reports on the conformational analysis of the 14-membered macrolides, erythromycin and oleandomycin,[66-69] and these macrolides are known to take one stable conformation. In the case of the 16-membered macrolide, leucomycin, conformation of the aglycone portion was examined by IR, CD, and NMR spectral analyses and from X-ray diffraction data of demycarosyl-isoleucomycin A_3 (**49**). In contrast to the former, this macrolide was found to have a flexible conformation.[70] CD measurements in various solvents and under variable temperature have shown that in the lactone, the ester-carbonyl region is especially mobile and the NMR spectral analysis has revealed that the CHO group, acetyl group in 3-position, and the proton in the 11-position are all in close proximity above the lactone ring. In contrast to the stable conformation of erythromycin, that of leucomycin is highly mobile and this may be due to the absence of conformational stabilization by the many methyl groups found in the former.

2. Chemical and Biological Transformation of 16-Membered Macrolides

Chemical transformation of 16-membered macrolides was carried out for their structural studies. Numerous new derivatives were discovered through chemical modification, *in vivo* metabolism, and microbial transformations. Some of the acylated antibiotics so obtained have been and are being used clinically.

(1) Acylation, Deacylation, and Hydroxylation

Taking leucomycin as an example of the 16-membered macrolides, its functional group, the hydroxyl, is present in 3, 9, 2′, 3″, and 4″ positions and these can be acylated with the exception of the tertiary hydroxyl in the 3″-position. Some of these hydroxyls are acylated in a natural state and many compounds have been obtained by chemical acylation. Comparison of various properties of the 10 components of leucomycin shows the effect of acyl group in the 3- and 4″-positions on the properties of the antibiotic. The antimicrobial activity of A_1 (**3**), A_5 (**6**), A_7 (**8**), and A_9 (**10**) which have a hydroxyl group in the 3-position of the lactone is generally higher than those of A_3 (**4**), A_4 (**5**), A_6 (**7**), and A_8 (**9**) having an acetyl group in the same position. However the latter shows a higher blood level and lower toxicity than the former. The antibacterial activity[71] and antimycoplasma activity[72,73] increase with increasing size of the acyl group in the 4″-position, from acetyl, propionyl, butyryl, to isovaleryl. Following the examples of acetylspiramycin,[72,74] and acetylation of tylosin (**18**),[75] the improvement of natural 16-membered macrolides by acylation has been attempted one after another, and numerous acyl derivatives have been prepared to date.

The blood level of 16-membered macrolides is said to be lower than that of 14-membered macrolides but this point has been remedied by acylation, as reported for acylated leucomycins,[76,77] propionyl-maridomycin,[78,79] propionyl-josamycin[80] (=9-propionyl-leucomycin A_3), and acetyl SF-837.[77] Derivatives with an acyl group in the 9-position are stable and have better antimicrobial activity than those with acyl group in the 2′-position. Two methods have been reported for preparing 9-acylated compounds. The one we reported involves diacylation of the 9- and 2′-positions, followed by removal of the acyl group in 2′-position by solvolysis.[71] The other, reported by OMOTO and others,[77] and by KISHI and his co-workers,[78] is treatment of the macrolide with acid chloride at a low temperature, in the presence of an amine, to introduce the acyl group directly into the 9-position (Chart 6).

Chart 6. The selective acylation of the 9-hydroxyl group in 16-membered macrolides

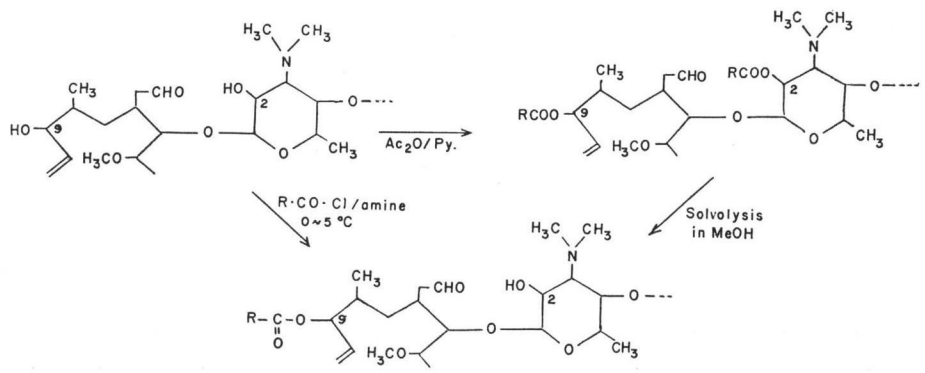


Table 3. Biological transformation of 16-membered macrolides

Transformation	Original antibiotics	Organisms	Reference
Deacylation of 4″-acyl	Leucomycin	*Diaporth* sp. *etc.*	83
		Mucor sp. *etc.*	87
		Bacteria	84
	Maridomycin	*Streptomyces* sp.	85
	Propionyl maridomycin	Rat liver homogenate	86
	Josamycin	Bacteria	84
	Niddamycin	*Streptomyces* sp.	87
		Cunning hamella and *Penicillium* sp.	
	Antibiotic SF-837	*Mucor* sp.	88
		Streptomyces sp.	
		Trichosporon	
	9-Acyl-SF-837	Rat liver homogenate, *Mucor. aspergillus*	89
		Crytococcus, Trichosporon etc.	
	Magnamycin A, B	Rat and chicken liver	90
	YL-704W, etc.	enzyme	91
Hydroxylation at β-position on isovaryl	Maridomycin	*Streptomyces olivaceus* 219	92
	Josamycin	〃	92
	9-Propionyl-Josamycin	Metabolites in man	93
Hydroxylation at C-14 on lactone	Antibiotic SF-837	Rat liver homogenate	94
	Josamycin	Metabolites in man	93
Reduction aldehyde	Tylosin	*Streptomyces* and *Nocardia*	95
	Maridomycin	*Nocardia mexicana* IFO 3927	96

The acyl group in the 4″-position of mycarose plays an important role in the antibacterial activity[71] and antimycoplasma activity[73] of the macrolide, and there are many reports on the deacylation of this position by microorganisms or with animal liver homogenate in order to exchange this acyl with another acyl group. Removal of the acyl group from the 4″-position results in the lowering of the antibacterial activity but introduction of a new acyl group into this position regenerates a better antibacterial activity.[81] YAMAGUCHI and others obtained new derivatives with excellent antibacterial activity by benzoylation of the hydroxyl group in the mycarose moiety.[81] Thus, the acyl group in the mycarose moiety plays an important part in the antibacterial activity of the macrolide but it is easily hydrolyzed during metabolism *in vivo*, and this is one of the reasons why the antibacterial activity of 16-membered macrolides does not last a long period of time. KAWAHARA and others[82] found that the introduction of a hydroxyl group into the position β to the isovaleryl group by an enzyme system produced by *Streptomyces olivaceus* 219 resulted in the improvement of not only in relation to the above point but also its solubility in water, leading to improved transport into the organs. Some 16-membered macrolides undergo enzymic hydroxylation at the 14-position *in vivo*, resulting in lowered antibacterial activity,[92-94] and this change is seen in the antibiotics SF-837 and josamycin, which have a diene conjugation in the 10—11 and 12—13 position, and not in marido-

mycin, which has an epoxide linkage in the 12—13 position, suggesting substrate specificity of the enzyme involved. Rosamicin (**40**) and cirramycin A_1 (**22**), which have a methyl group in this 14-position, are interesting antibiotics in this connection.

There have been many reports on the biological deacylation of 16-membered macrolides (Table 3) but there has not been found any microorganism reported which causes deacylation of the acyl group in 3-position of the lactone.

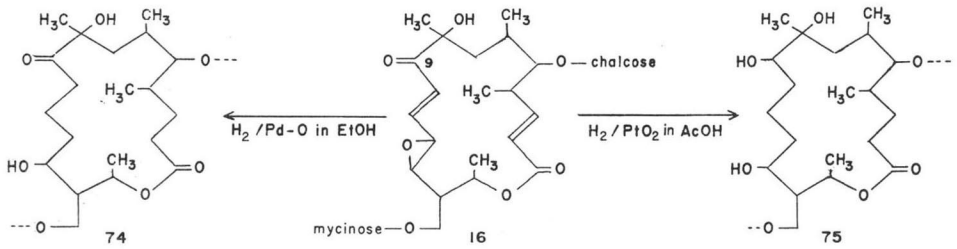
TSUKIURA and others examined the biological properties of many derivatives of cirramycin A_1 (**22**).[97] Among these derivatives, 2′,4′-disuccinyl-cirramycin A_1 (**73**) was found to possess some improved biological properties with respect to oral absorptivity, local tissue irritability, and general toxicity. This modification is of interest in that the macrolide was converted to an amphoteric substance.

(2) Modification of Aldehyde

Chemical and biological modification of the aldehyde group, common to basic 16-membered macrolides, has been made on many of the antibiotics and the effect of this functional group on their antibacterial activity has been examined. Activity of the compounds obtained by chemical reduction of this group in leucomycin[71] and spiramycin[98,99] was examined. Maridomycin, 9-propionylated maridomycin, and josamycin[96] were transformed to their 18-dihydro compounds by the action of *Nocardia mexicana* IFO 3927. In all these cases, reduction of the aldehyde group resulted in a marked reduction of the antibacterial activity (MIC) *in vitro*. When the aldehyde group in leucomycin A_3 (**4**) was converted to an alcohol or thiosemicarbazone, its activity decreased markedly, but conversion into hydrazones led to retention of the activity in some cases. The leucomycin hydrazone of 1-amino-4-methyl-piperazine exhibited almost the same antibacterial activity as leucomycin, and showed an improved blood level.[76]

Macrolides with 12- to 16-membered ring possess a carbonyl group in the aglycone which is important for their biological activity. This is the formylmethyl group bonded to the 6-position of the lactone in the 16-membered macrolides. Chalcomycin (**16**) and neutramycin

Table 4. Antimicrobial activity of chalcomycin derivatives



Test organisms	MIC*(μg/ml)		
	16	**74**	**75**
Bacillus subtilis PCI 219	25	50	>100
Staphylococcus aureus FDA 209P	0.8~3.12	1.56	>100
Sarcina lutea PCI 1001	0.2	0.2~12.5	25
Mycoplasma hominis type IC	3.13	50	>100

* Agar dilution streak method.

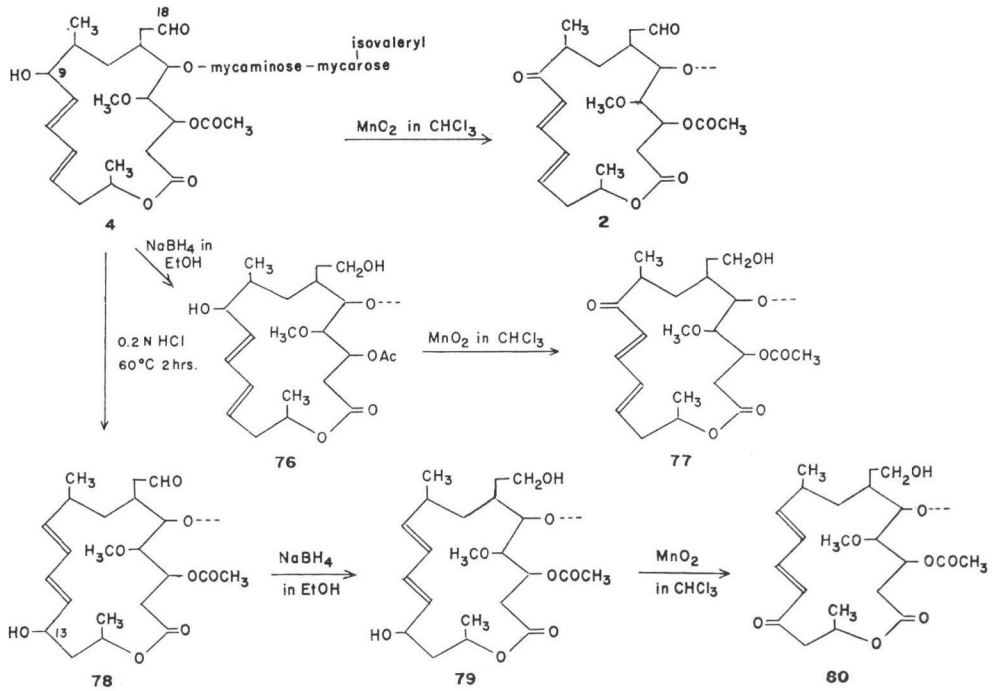
(**17**), neutral 16-membered macrolides, do not have this formyl group but have a carbonyl group in the 9-position. Contribution of this carbonyl group in chalcomycin (**16**) to its antibacterial activity was found from the comparison of minimum inhibitory concentration (MIC) of the two reaction products, tetrahydro- (**74**) and hexahydro-chalcomycins (**75**). Reduction of the double bond and the epoxide does not affect the antibacterial activity but reduction of the carbonyl group results in the disappearance or great reduction in this activity (Table 4).

On the other hand, leucomycins do not have a carbonyl in the 9-position but do have an aldehyde group characteristic of 16-membered macrolides. Comparison of the antibacterial activity[100] of a series of compounds obtained by modification of the aldehyde and 9-hydroxyl group in this 16-membered macrolide revealed the contribution of the aldehyde group or, in its place, the carbonyl group in the 9-position, on the antibacterial activity of these macrolides (Table 5).

(**3**) Allylic System at C-9 to C-13

Reduction of the chromophore groups in C-9 to C-13 positions common to basic 16-

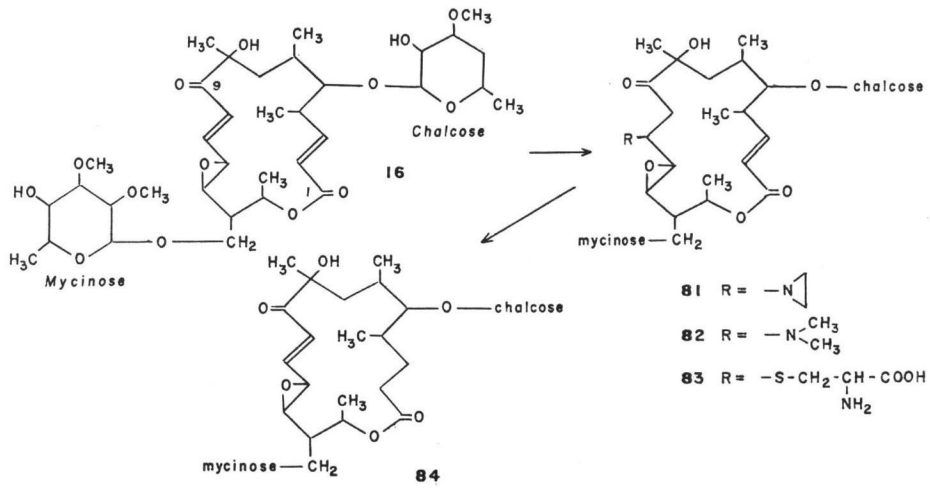
Table 5. Antimicrobial activity of leucomycin derivatives



Test organisms	MIC*(μg/ml)						
	4	**2**	**76**	**77**	**78**	**79**	**80**
Bacillus subtilis PCI 219	0.2	0.4	>100	25	0.05	>100	50
Staphylococcus aureus FDA 209P	0.4	0.4	>100	25	0.05	>100	100
Sarcina lutea PCI 1001	0.05	0.05	100	1.56	0.05	>100	6.25
Mycoplasma hominis type IC	<0.20	—	>100	1.56	<0.20	100	25

* Agar dilution streak method.

Chart 7. Chemical modification of chalcomycin



membered macrolides, gives compounds with comparable antibacterial activity (MIC) as that of parent compound.[71,98,99]

Leucomycin and SF-837 possess an allyl alcohol system in C-9 to C-13 positions and undergo isomerization to 13-hydroxy compounds by allyl rearrangement in acid reaction (pH 2.5~3.8).[18,19,101] The antibacterial activity of the compounds so obtained is the same as or slightly lower than that of the parent compounds. It seems impossible to consider, from these results, that the groups in the C-9 to C-13 positions have any direct effect on the antibacterial activity in these macrolides.

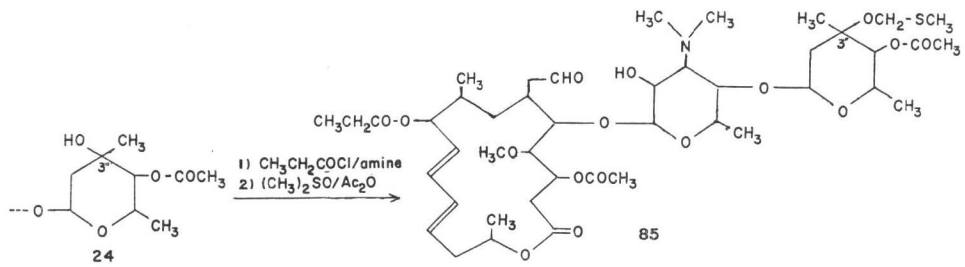
Chalcomycin (**16**) has been modified by the selective MICHAEL-type addition of amines and thiols to its α,β-unsaturated ketone to the exclusion of the α,β-unsaturated lactone[102] (Chart 7). Compounds substituted in the C-11 position so obtained were 11-(1-aziridinyl)-10, 11-dihydrochalcomycin (**81**), 11-(dimethylamino)-10,11-dihydrochalcomycin (**82**), and 11 [(L-2-amino-2-carboxyethyl) thio]-10,11-dihydrochalcomycin (**83**). In addition, 2,3-dihydrochalcomycin (**84**), synthesized by a hydrogenation-elimination sequence of reactions starting with chalcomycin (**16**), was obtained and the antibacterial activity of these compounds was examined. Essentially all of the modified compounds had approximately the same ability as the parent chalcomycin (**16**) against *Staphylococcus aurues in vitro*. *In vivo* activity against *Staph. aureus* comparable to that of chalcomycin (**16**) was demonstrated in from 11-(1-aziridinyl)-10,11-dihydrochalcomycin (**81**) to 11-[(L-2-amino-2-carboxy ethyl) thio]-10,11-dihydrochalcomycin (**83**).

The aglycone of chalcomycin (**16**) and neutramycin (**17**) possesses an α,β-unsaturated lactone, and the deaceticacid compound[103] of leucomycin that corresponds to it has no antibacterial activity.[104] This is evidence that the structure-biological activity correlation in 16-membered macrolides is quite complicated.

(**4**) Modification of Sugars

Modification of the mycarose moiety is mostly centered at its 4″-position and many reports on it have been published, as stated earlier. INOUYE and his co-workers[105] obtained thiomethoxymethyl derivatives. Derivatives were made of the tertiary hydroxyl group in the 3″-

Table 6. Antimicrobial spectra of SF-837 A_1 (**24**) and 9-propionyl-3″-thiomethoxymethyl SF-837 A_1 (**85**)



24 85

position by treatment of acyl derivatives of SF-837 A_1 (**24**) with dimethyl sulfoxide and acetic anhydride. One such derivative, 9-propionyl 3″-thiomethoxymethyl SF-837 A_1 (**85**), was reported to have higher antimicrobial activity than the parent compound against *Escherichia coli.*, *Mycobacterium*, and *Mycoplasma*, including a tylosin-resistant strain, *in vitro*. This compound also showed a better effect than the parent SF-837 A_1 (**24**) in the treatment of experimental infection of mice with *Staph. aureus* 209P[105] (Table 6).

Test organisms	MIC (μg/ml)	
	24	**85**
Escherichia coli	100	50
Mycobacterium 607	25	6.25
Staphylococcus aureus	0.78	0.78
Neisseria gonorrhoeae Megurita	25	100
Neisseria meningitidis	0.78	6.25
Mycoplasma gallisepticum S-6	0.1	<0.1
〃 S-15P	0.05	<0.1
〃 S-35P	0.05	<0.1
〃 T-4AT	>100	125
〃 CH^3T	<0.05	<0.1
〃 KP-3	<0.05	<0.1
〃 KP-13	<0.05	<0.1
Vibrio coli 34E	125	25
〃 SD-358	>50	>50
〃 SD-362	>50	25

There are no examples of formation of a new compound with good antimicrobial activity by chemical modification of mycaminose but some of the compounds so obtained are considered to be important in determining the relationship between chemical structure and biological activity of these antibiotics. Removal of the *N*-methyl group from magnamycin with iodine or bromine has been reported by FREIBERG.[106] We obtained 3′-de(dimethylamino) 3′-oxo compound (**88**) and 3′-*N*-demethyl-*N*-acetyl compound (**89**) by reacting leucomycin A_3 (**4**) with *m*-chloroperbenzoic acid to change it to its *N*-oxide (**87**) and refluxing the oxide compound with acetic anhydride in chloroform.[107] Oxidation of leucomycin A_3 (**4**) with chromium trioxide-pyridine complex gave the *N*-formyl compound (**90**).[108] A series of these chemical modifications resulted generally in compounds with decreased antibacterial activity (Chart 8).

As stated earlier, rosamicin (**40**)[61] is the only 16-membered macrolide that possesses desosamine but its antimicrobial activity is approximately the same as that of other macrolides of this group and this fact indicates that the hydroxyl group in the 4′-position of mycaminose is not essential for antibacterial activity. Also as stated earlier, angolamycin (**20**)[35] has a deoxyamino sugar, angolosamine, that lacks 2′-OH in mycaminose but this macrolide also possesses antibacterial activity, indicating that the hydroxyl in the 2′-position of mycaminose is not very important for antibaterial activity. These facts are interesting in that they differ from the evidence found in 14-membered macrolides, erythromycin and kujimycin, whose

Chart 8. Chemical modification of mycaminose

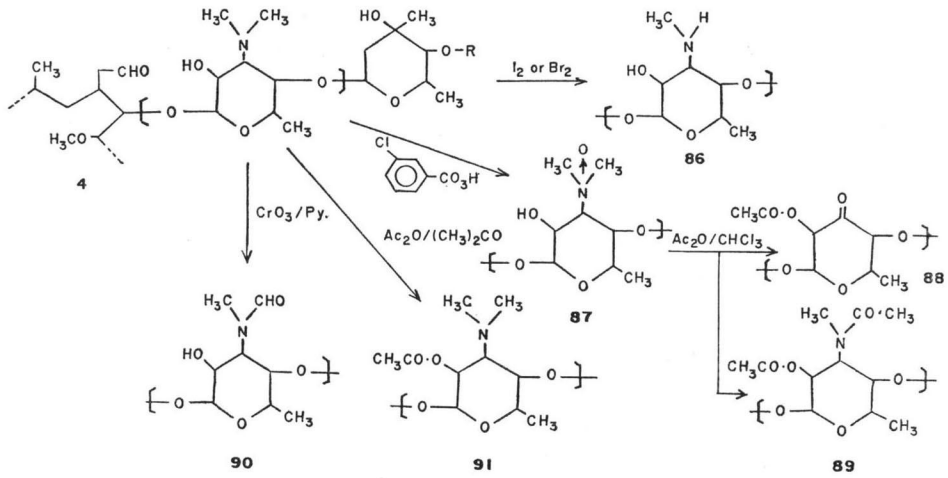
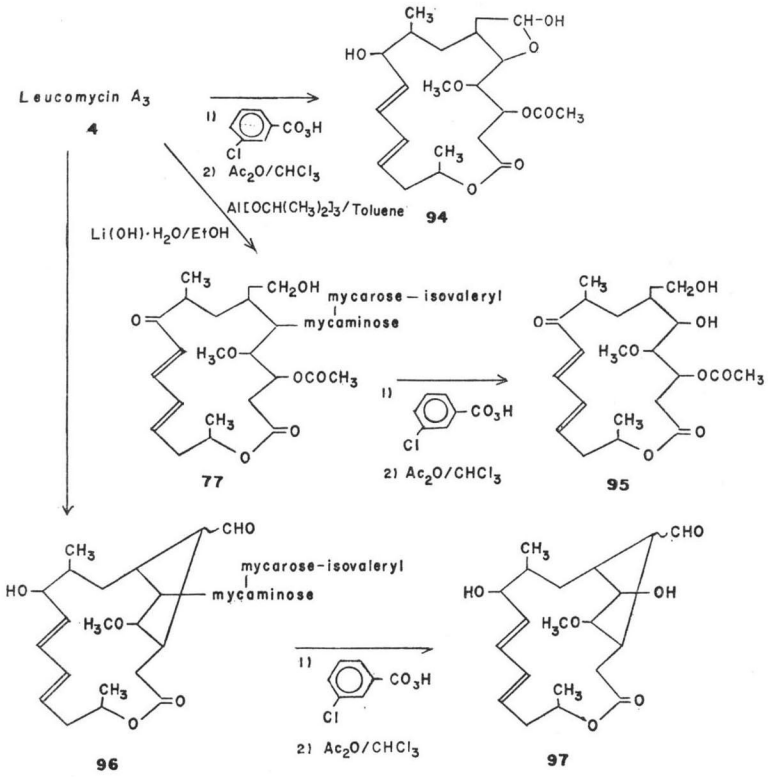


Chart 9. Isolation of aglycone



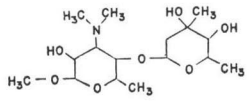
antibacterial activity is markedly decreased or lost by acetylation of the hydroxyl in the deoxysugars, desosamine and chalcose, bonded to the 5-position of the lactone and the report that this hydroxyl is important for antibacterial activity of these macrolides.[109,110]

(5) Isolation of Aglycone

It is important to obtain the lactone portion, the aglycone of 16-membered macrolides, not only for studies on the relationship between structure and biological activity, but also for

studies on the biosynthesis of these macrolides, and for structural studies on structurally unknown macrolides. Many attempts have been made to remove mycaminose, the amino sugar, from the lactone portion, but none of them have proved to be practicable. For example, MORIN and his co-workers[80] treated tylosin (**18**) with an acid, but reported that the yield of the aglycone was extremely low. SUZUKI and his co-workers[111] also obtained the aglycone in a low yield by de-epoxidation of antibiotic B-58941 (**23**) and refluxing its product with a sulfonic acid-type ion-exchange resin. Both these aglycones do not retain the original lactone structure. FURUMAI and his co-workers[112] reported platenolide I (**92**) and II (**93**), the modified aglycone of platenomycin, obtained by the blocked mutants of *Streptomyces platensis* subsp. *malvinus*. As shown in Chart 9, we have found a new method to remove mycaminose from the lactone in a good yield by deriving leucomycin A_3 (**4**) to its *N*-oxide (**87**) with *m*-chloroperbenzoic acid and refluxing the oxide compound with acetic anhydride in chloroform.[113] Leuconolide A_3 5, 18-hemiacetal (**94**) was separated from leucomycin A_3 (**4**) by such a method, and the lactone portion, 9-dehydro-18-dihydro-leuconolide A_3 (**95**) having a carbonyl in the 9-position, was also isolated due to the carbonyl-hydroxyl stero-isomer mentioned earlier. The deacetoxylated compound (**96**) obtained by refluxing leucomycin A_3 (**4**) with lithium hydroxide monohydrate in ethanol, and the aglycone 3-deacetoxyl-3,6-bicycloleuconolide A_3 (**97**), having a bicyclic lactone structure, was isolated by the same method.[114]

Fig. 8. Structure of methyl-4-*O*-(L-mycarosyl)-β-D-mycaminoside (**98**)



The disaccharide in leucomycin U (**11**), methyl-4-*O*-(L-mycarosyl)-β-D-mycaminoside (**98**), was synthesized and its antibacterial activity was examined along with that of the aglycone portion[115] (Fig. 8). Neither compound had any biological activity and this demonstrated that at least the lactone plus amino sugar moiety is necessary for the antibacterial activity in these macrolides.

(6) Structure-activity Relationship Examination Using Cell-free System

We have been able to synthesize many derivatives of leucomycins and, for further evaluation of structure-activity relationships, examinations were made on the binding of these derivatives to ribosomes, taking into consideration the permeability of these compounds through the cell membrane.

Macrolide antibiotics in general are known to bind with the 50S subunit of prokaryote ribosomes and to inhibit biosynthesis of the peptide.[116] There is a specific binding of erythromycin to ribosome in a 1:1 ratio under certain conditions but, in the presence of erythromycin derivatives, the derivative takes the place of erythromycin and the replacement reaches an equilibrium.[117~119] The ratio of this replacement was observed to parallel the antibacterial activity of these derivatives. We examined the concentration of leucomycins that inhibited the binding of ^{14}C-erythromycin with ribosomes to 50% of the control, and compared these values with the *in vitro* antibacterial activities of leucomycins and their derivatives.[120] The binding of ^{14}C-erythromycin to ribosomes was examined by varying the concentration of the leucomycins from 10^{-7} to 10^{-8} molarity. Table 7 shows a comparison of the concentration of various derivatives for 50% inhibition of the binding of ^{14}C-erythromycin to the ribosomes and the minimum inhibitory concentrations (MIC) of the derivatives against *Bacillus subtilis*,

Table 7. Antimicrobial activities of leucomycins and derivatives

Compounds		Minimum inhibitory concentration (μg/ml)			Concentration of 50% inhibition of ^{14}C-erythromycin binding to ribosomes μmol
		B. subtilis PCI 219	*S. aureus* FDA 209P	*E. coli* NIHJ	
Leucomycin-A_5	**6**	0.2	0.1	12.5	1.8
Leucomycin-A_1	**3**	0.2	0.1	12.5	2.1
Leucomycin-A_4	**5**	0.4	0.2	25	2.6
Leucomycin-A_6	**7**	0.78	0.4	100	3.5
Leucomycin-A_3	**4**	0.2	0.2	25	4.2
Magnamycin-B	**2**	0.4	0.4	25	4.2
Leucomycin-A_5	**9**	1.6	1.6	100	14
Leucomycin-U	**11**	6.25	6.25	200	21
Leucomycin-A_3 N-oxide	**87**	50	100	>200	30
Demycarosyl-leucomycin-A_3	**48**	50	25	>200	91
9-Dehydro-18-dihydro-leucomycin-A_3	**77**	25	25	>200	123
18-Dihydro-leucomycin-A_3	**76**	200	>200	>200	209
2′-O-Acetyl-3′-dedimethylamino-3′-oxoleucomycin-A_3	**88**	100	100	>200	288
Leuconolide-A_3 5,18-hemiacetal	**94**	>200	>200	>200	309
3-Deacetoxy-3,6-bicyclo-leucomycin-A_3	**96**	>200	>200	>200	447
9-Dehydro-18-dihydro-leuconolide-A_3	**95**	>200	>200	>200	3,160

Staph. aureus, and *E. coli*. In can be seen that the compounds having a small MIC value on the upper part of this table also have a low value for the 50% inhibitory concentration. The antibacterial activity of these compounds correlated well with their ability to bind with ribosomes. It is thought that this kind of physical measurements of compounds obtained by modification of natural macrolides and analysis of the results can be an important means, not only for obtaining more useful compounds but also for elucidation of the binding mechanism of macrolides with ribosomes.

RAKHIT and SINGH[121] compared the antibacterial activity and protein synthesis inhibition of 18 kinds of compounds obtained by chemical transformation of leucomycins at the C-9 to C-18 positions and the aldehyde group. Antibacterial activity determined with *B. subtilis* and *Staph. pyogenes* and inhibition of protein synthesis by the incorporation of ^{14}C-leucine into *B. subtilis* and poly-U-directed phenylalanine incorporation into the cell-free system from *E. coli* was used as an index. Their report indicated the necessity of such a comparison in discussing the correlation of structure and activity taking into consideration the transport of such compounds into the cells.

(7) Biosynthesis of 16-Membered Macrolides

Biosynthesis of 16-membered macrolides, especially of magnamycin (**2**), has attracted the interest of many workers. To date, it has been revealed that the sugars, mycaminose and mycarose, originate from glucose, that the *N*-methyl on mycaminose and *O*-methyl in the lactone originate from the *S*-methyl group of methionine, and that the isovaleryl group on mycarose has L-leucine as its precursor.[122]

Some hypotheses have also been presented for the skeleton of the lactone.[122] GRISEBACH and ACHENBACH[123] measured the radioactivity of magnamycin (**2**) and its oxidation products (**99**～**101**) after incorporation of acetate [1-^{14}C] and acetate [2-^{14}C] and reported that there was no incorporation of the acetate into carbon atoms at the 5—6 and 17—18 positions. GRISEBACH concluded, later, that the precursor of these carbon atoms is a compound related to sugar metabolism other than the acetate, such as succinate.[124]

SRINIVASAN and his co-workers[125,126] used a similar technique for the incorporation of acetate [2-^{14}C] into magnamycin (**2**) and found that the radioactivity of C_{12} acid (**100**) (Chart 10) so obtained was 40 % of carimbose (**99**), indicating that this acetate is incorporated into the 12- to 18-positions in the same ratio. They concluded that the aldehyde and the methylene (C-18 and C-17) originated from the acetate. GRISEBACH[127] later reexamined the origin of the aldehyde group and revealed that the radioactivity of carbon dioxide from the DIECKMANN reaction product of the C_8 acid (**101**) derived from radioactive magnamycin (**2**), obtained by the addition of succinate [2, 4-$^{14}C_2$], was only 3 % of that of magnamycin (**2**). This fact indicates that the hypothesis proposed by him that the aldehyde group originates in succinate is unlikely.

Recently, ^{13}C-NMR spectroscopy has been shown to be useful for the elucidation of biosynthetic pathways of microbial metabolites.[128~130] The advantage of this method lies in the ability to assign unequivocally the locus of incorporation of ^{13}C isotopic excess in the intact metabolite rather than its degradation products, as must be done with ^{14}C-labelled materials. Biosynthesis of leucomycins has been examined with the use of ^{13}C-labeled precursors such as acetate [1-^{13}C], acetate [2-^{13}C], acetate [1, 2-$^{13}C_2$], propionate [1-^{13}C], butyrate [1-^{13}C] and succinate [1, 4-$^{13}C_2$].[131] Results of these examinations supported past results that carbon atoms at 1, 2, and 9 to 16 positions are derived from acetate but incorporation of acetate into carbon atoms at 3 to 6, 17, and 18 positions was so small that it would be difficult to consider acetate as their precursors. From the feeding experiment of ^{13}C-1-butyrate it was concluded that four carbons, 5, 6, 17 and 18 were derived from butyrate.[131] This raises the doubt about the conclusion of SRINIVASAN and his co-workers[126,127] that the aglycone portion of magnamycin (**2**) is biosynthesized from eight acetates and one propionate.

FURUMAI and his co-workers[112] obtained many blocked mutants from platenomycin (PL) producing strain by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and/or ultraviolet irradiation, and obtained five kinds of intermediates in the biosynthesis of platenomycin produced by these mutants; platenolide-I (**92**), platenolide-II (**93**), 3-*O*-propionyl-5-*O*-mycaminosyl-platenolide-I (**104**), 3-*O*-propionyl-5-*O*-mycaminosylplatenolide-II (**105**), and demycarosylplatenomycin (**106**). By using the washed mycelium of these mutants, they showed that platenolide I (**92**) is incorporated into platenomycins (**24**, **29**, and **31**) by the route shown in Chart 11. It should be noted that the aldehyde group in platenomycins was formed by the oxidation of a methyl group.

ONO and his co-workers[132] examined the incorporation of several ^{14}C-labeled compounds into maridomycin by *Streptomyces hygroscopicus*, and found that L-methionine [methyl-^{14}C], propionate [1-^{14}C], propionate [2-^{14}C], propionate [3-^{14}C], acetate [U-^{14}C], butyrate [1-^{14}C], propanol [1-^{14}C], glycerol [U-^{14}C], L-leucine [U-^{14}C], and isoleucine [U-^{14}C] were incorporated into maridomycins to a significant extent. These data are not inconsistent with the known

Chart 10. Degradation of magnamycin

2 = Magnamycin, **99** = Carimbose, **100** = C_{12} acid, **101** = C_8 acid, **100** = Dieckmann product

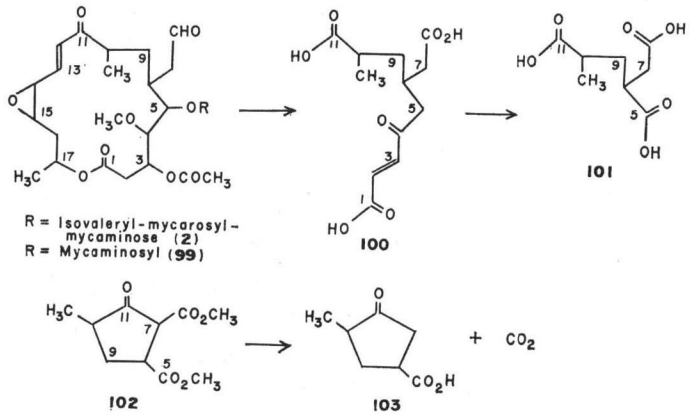
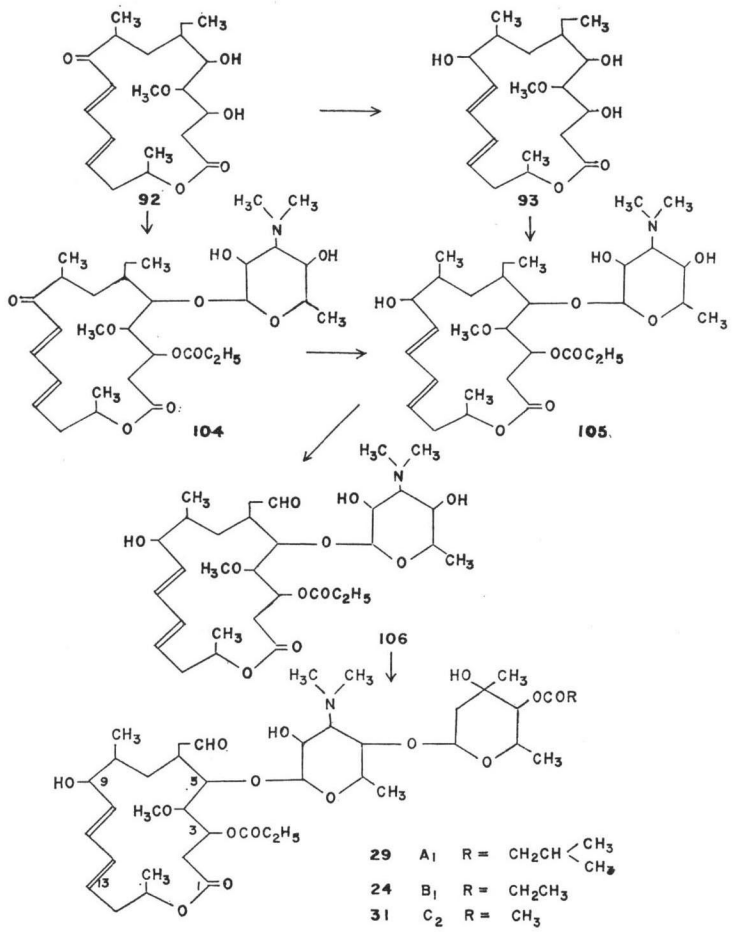


Chart 11. Biosynthetic pathway of platenomycins



results on the biosynthesis of magnamycin, and the fact that butyrate [1-^{14}C] was incorporated into the aglycone portion of maridomycin in the same way as propionate [3-^{14}C] indicates, together with the incorporation experiment of FURUMAI and his co-workers with the intermediates, that there is a strong possibility for the origin of the aldehyde in the methyl of the butyryl group.

PAPE and BRILLINGER[133] found, by the use of a cell-free extract obtained from the cells of tylosin-producing strain of *Streptomyces rimosus*, that thimydine diphosphate (TDP)-L-mycarose (**109**) is biosynthesized from TDP-D-glucose (**107**) and *S*-adenosyl-L-methionine, in the presence of NADPH, via the intermediate TDP-4-keto-6-deoxy-D-glucose (**108**) (Chart 12). This fact indicates that mycarose is not biosynthesized after glucose or its derivative has bonded with the mycaminose portion but that mycarose is biosynthesized separately and then binds with mycaminose. Consequently, correlation between mycarose and the deoxy-sugar, 2, 3, 6-trideoxy-L-pyranosyl-4-ulose (**110**) seen in the antibiotic, B-58941 (**23**), reported by SUZUKI and others,[42] in connection with its biosynthesis is an interesting problem left for future elucidation.

Early studies on the biosynthesis of magnamycin and recent studies on the biosythesis of 16-membered macrolides are illustrated in Chart 13.

Chart 12. Possible mechanism of mycarose biosynthesis

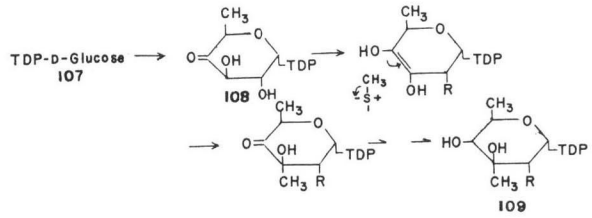


Chart 13. The mechanism of the biosynthesis of magnamycin $B_4^{\pm}$(**2**)

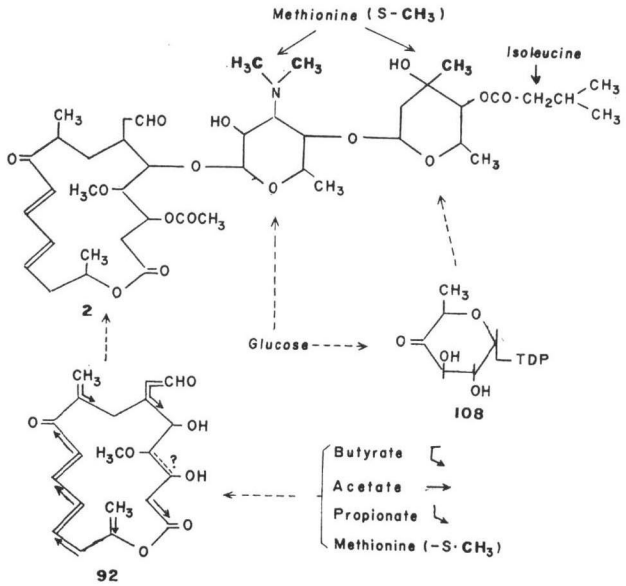
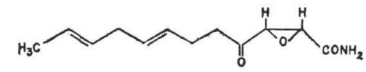


Fig. 9. Structure of cerulenin (**111**)



We have recently found that the antifungal antibiotic, cerulenin (**111**) (Fig. 9), inhibits biosynthesis of fatty acids and that this action involves the so-called condensation enzyme which takes part in the condensation of acetate and malonate.[134,135] It was also found that cerulenin inhibit the biosyntheses of leucomycin, cycloheximide, and tetracycline which are formed from "polyketide" derived from the condensation of acetate and malonate (or methylmalonate).[136] Similarly, biosyntheses of polyene macrolides,[137] 6-methylsalicylic acid,[138] and chalcone in the cell-free system are inhibited by cerulenin (**111**), and it is likely that this reagent will increasingly be used for studies on the biosynthesis of natural products.

Fermentation of 16-membered macrolides is an interesting subject in connection with their biosynthesis but there are few report on it. For example, addition of 0.1 mg/ml of tylosin hydrolyzate or 0.3 mg/ml of *O*-mycaminosyltylonolide (**112**) to the medium at the start of the fermentation of tylosin (**18**) was found to raise the yield of tylosin (**18**) to 5.34 and 6.02 mg/ml, respectively, in contrast to 4 mg/ml produced without their addition.[139] Increased production of carbomycin, from 1,715 to 2,073 mg/ml, by the addition of 40~65 ppm of Ca^{++} to the medium of *Streptomyces halstedii* was reported.[140] Examination of the effect of organic acids (acetate, propionate, butyrate, valerate, methyl malonate, and ethyl malonate) on the fermentative production of leucomycin showed that production of the antibiotic was increased 1.5~2 fold by the addition of butyrate and ethyl malonate.[141] KODAMA and his co-workers[142] examined the effect of aeration and agitation on the fermentative production of leucomycin.

Chemical and biological studies on 16-membered macrolides have suddenly become very active in recent year but there are still numerous problems remaining unsolved. With respect to their biosynthesis, some primary precursors have not been elucidated. There are interesting and yet unsolved problems such as the action mechanism and relation of the structure to the induction of resistance to macrolides. Although numerous derivatives have been prepared from these macrolides there is still no derivative that is effective against gram-negative bacteria or to macrolide-resistant bacteria. These problems are left for future elucidation, together with the discovery of many new macrolides anticipated in the years to come.

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