THE CHEMISTRY OF LEUCOMYCINS. VI

STRUCTURES OF LEUCOMYCIN A_4, A_5, A_6, A_7, A_8 AND A_9

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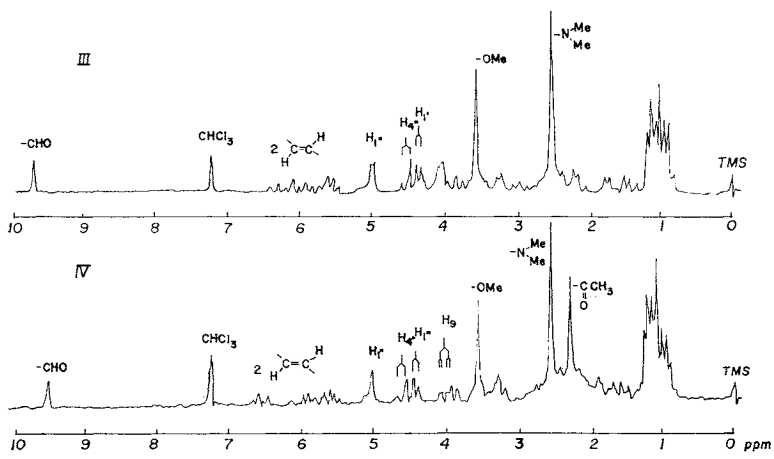
Studies on the structures of new components of leucomycin, A_4(IV), A_5(III), A_6(VI), A_7(V), A_8(VIII) and A_9(VII), were carried out in parallel with those on components A_1(I) and A_3(II), namely acetylation, and acid and alkaline hydrolysis. The pairs of components IV and III, VI and V, and VII and VIII gave respectively, acetates X, XI and XIII. Components IV, VI and VIII had then respectively, 4-O-*n*-butyryl-, 4-O-propionyl- and 4-O-acetyl-mycarose replacing the 4-O-isovaleryl mycarose in the A_3. Like A_3, components IV, VI and VII had an O-acetyl group on C-3 of the 16-membered lactone.

Leucomycins A_4, A_5, A_6, A_7, A_8 and A_9, as reported earlier, are new components of leucomycin produced by an actinomycetales, *Streptomyces kitasatoensis* HATA[1,2]. The structures of these new components were the subject of a brief communication[3]. The present paper presents the details for the communication plus some spectroscopic features of the degradation products of these components.

The UV spectra of these components showed absorption maxima at 232 mμ, which indicates the presence of the same chromophores as in leucomycin A_3(II)[4,5].

Although the NMR spectra of these components are quite complex, with many overlapping areas, they provide some valuable information. The similarities in the NMR spectra of leucomycin A_3(II), A_4(IV) $C_{41}H_{67}O_{15}N$ (Fig. 1), A_6(VI) $C_{40}H_{65}O_{15}N$, and A_8(VIII) $C_{39}H_{63}O_{15}N$ in the 3.5~10 ppm region can be seen together with the

Fig. 1. NMR spectra of leucomycin A_5 (III) and leucomycin A_4 (IV) (100 Mc, in $CDCl_3$)

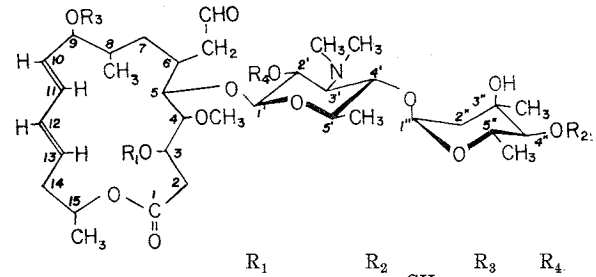


obvious differences at higher field, especially in the C-methyl region. In the spectra of four components the characteristic O-acetyl peak at 2.22 ppm was observed. On the other hand, the similarities of the spectra of components A_1(I), A_5(III) $C_{39}H_{65}O_{14}N$ (Fig. 1), A_7(V) $C_{38}H_{63}O_{14}N$, and A_9(VII) $C_{37}H_{61}O_{14}N$ also can be seen in the 3.5~10 ppm region together with obvious differences in the C-methyl region. No O-acetyl singlet at 2.22 ppm was observed in these spectra, and only component A_9(VII) showed an O-acetyl peak at 2.05 ppm. Some of the major signals in the NMR spectra of components A_5(III) and A_4(IV) can be assigned as shown in Fig. 1. It has been reported that when the hydroxyl group at the C-3 position of the lactone of leucomycin A_1(I) was replaced by O-acetyl, some striking differences, especially in the 4.5~10 ppm region, were observed in the NMR spectra.

From the NMR data, it is suggested that the structures of components III, V and VII are very similar to that of I[6,7], while on the other hand, components IV, VI and VIII are similar to II. In order to resolve this, parallel experiments were carried out on these components and with components A_1(I) and A_3(II). From the results the similarity was substantiated. By subsequent work, the complete structural relationship was elucidated (Chart 1).

On the alkaline hydrolysis of III, V and VII, one mole of a volatile acid was eliminated. On the other hand, two moles of a volatile acid were eliminated from IV, VI and VIII. On acetylation with acetic anhydride in pyridine, components III, V and VII were converted to the corresponding triacetates, while IV, VI and VIII gave diacetates. Comparing diacetyl leucomycin A_4(X) $C_{45}H_{71}O_{17}N$ with triacetyl leucomycin A_5 by NMR spectra in $CDCl_3$ (100 Mc/s), IR spectra in CCl_4, and behavior on thin-layer chromatography, complete coincidence was found between both. Furthermore, these acetates were identical by mixed melting point. The same comparison between diacetyl leucomycin A_8(XII) $C_{43}H_{67}O_{17}N$ and triacetyl leucomycin A_9 proved them to be identical.

Chart 1



		R_1	R_2	R_3	R_4
Leucomycin	A_1(I)	H	$COCH_2CH(CH_3)_2$	H	H
//	A_3(II)	$COCH_3$	//	//	//
//	A_5(III)	H	$COCH_2CH_2CH_3$	//	//
//	A_4(IV)	$COCH_3$	//	//	//
//	A_7(V)	H	$COCH_2CH_3$	//	//
//	A_6(VI)	$COCH_3$	//	//	//
//	A_9(VII)	H	$COCH_3$	//	//
//	A_8(VIII)	$COCH_3$	//	//	//
	IX	//	$COCH_2CH(CH_3)_2$	$COCH_3$	$COCH_3$
	X	//	$COCH_2CH_2CH_3$	//	//
	XI	//	$COCH_2CH_3$	//	//
	XII	//	$COCH_3$	//	//
	XXIV	H	H	H	H

Chart 2

I, II $\xrightarrow{*}$ XI III, IV $\xrightarrow{*}$ X

V, VI $\xrightarrow{*}$ XI VII, VIII $\xrightarrow{*}$ XII

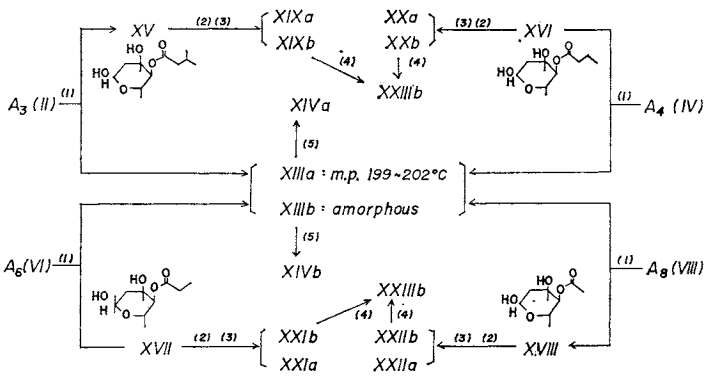
* Py-Ac_2O

Hydrolysis of component IV with 0.3 N hydrochloric acid afforded basic substances, $C_{30}H_{49}O_{11}N$ (m.p. 199~202°C) (XIIIa), $C_{30}H_{49}O_{11}N$ (amorphous) (XIIIb) and a new oily acyl mycarose (XVI). XVI was converted to its methyl

glycoside and subsequently separated into the α- and β-anomers; α-(XXa) and β-methyl-4-O-*n*-butyryl mycaroside (XXb), $C_{12}H_{22}O_5$, by silicic acid column chromatography[8]. By a similar method, components VI and VIII afforded new anomers, α-(XXIa) and β-methyl-4-O-propionyl mycaroside (XXIb), $C_{11}H_{20}O_5$, and α-(XXIIa) and β-methyl-4-O-acetyl mycaroside (XXIIb), $C_{10}H_{18}O_5$, respectively[9].

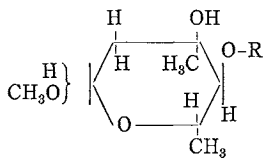
Hydrolysis of XXb, XXIb and XXIIb with 1 N sodium hydroxide gave the same methyl mycaroside (XXIV), $C_8H_{16}O_4$, as obtained from XIX[10,11]. The structures of these acyl glycosides were determined by means of Mass spectra (Fig. 2) and NMR spectra (Fig. 3).

Chart 3



(1) 0.3 N-HCl (2) 1% HCl·MeOH
(3) Chromatography (4) 1 N-NaOH (5) Py-Ac_2O

Table 1. Properties of methyl-4-O-acyl mycarosides and methyl mycarosides



		R	M. F. (M. W.)	Anal. % Calcd. C	Calcd. H	Found C	Found H	b. p. °C/mmHg	$[\alpha]_D^{25}$ (c 1.5, $CHCl_3$)	n_D^{25}
α	XIXa	-CO·CH_2·CH(-CH_3)-CH_3	$C_{13}H_{24}O_5$ (260.0)	59.98	9.29	60.08	9.18	115~116/2	−135.5	1.4476
β	XIXb					60.05	9.20	117~118.5/0.7	+ 13.5	1.4518
α	XXa	-CO·$CH_2CH_2CH_3$	$C_{12}H_{22}O_5$ (246.3)	58.52	9.00	58.61	8.98	112~122/3	−137.0	1.4481
β	XXb					58.72	8.95	118~119/2	+ 16.9	1.4512
α	XXIa	-CO·CH_2·CH_3	$C_{11}H_{20}O_5$ (232.3)	56.88	8.68	56.76	8.65	98~100/2	−145.0	1.4470
β	XXIb					56.80	8.70	109~110/2	+ 20.0	1.4515
α	XXIIa	-$COCH_3$	$C_{10}H_{18}O_5$ (218.2)	55.00	8.30	55.20	8.25	88~ 99/2	−148.0	1.4454
β	XXIIb					55.10	8.34	104~106/1.5	+ 25.0	1.4517
α	XXIIIa	-H	$C_8H_{16}O_4$ (176.0)	54.63	9.15	54.71	9.20	94~ 95/1.5 (m. p. 60°C)	−144.0	——
β	XXIIIb					54.65	9.25	108~112/1.5	+ 35.0	1.4545

Fig. 2. Mass spectra of β-methyl-4-O-*n*-butyryl mycaroside (XXb), β-methyl-4-O-propionyl mycaroside (XXIb) and β-methyl-4-O-acetyl-mycaroside (XXIIb)

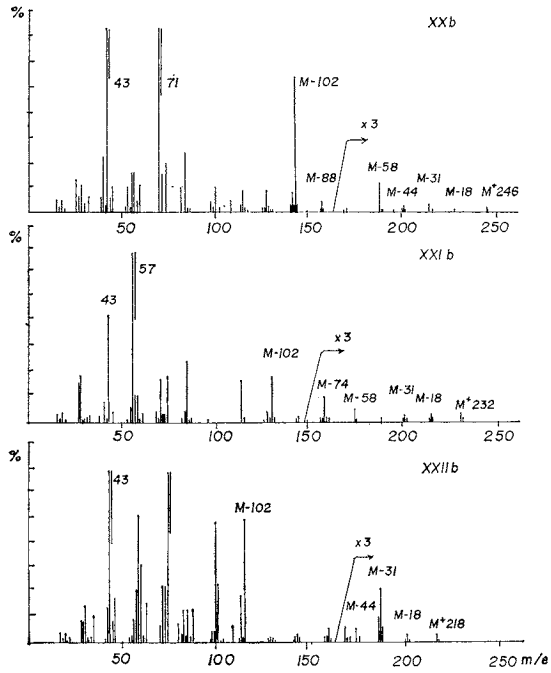
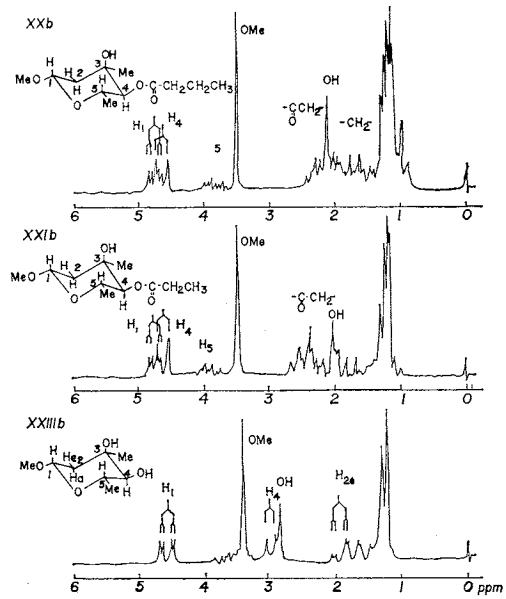
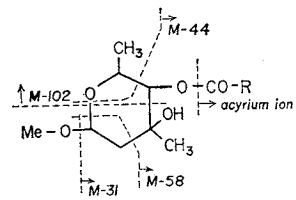


Fig. 3. NMR spectra of β-methyl-4-O-*n*-butyryl mycaroside (XXb), β-methyl-4-O-propionyl mycaroside (XXIb) and β-methyl-mycaroside (XXIIIb) (60 Mc, CCl_4)



As shown in the mass spectra of these acyl glycosides, besides the parent ion peaks and common characteristic peaks M-18 (M-H_2O), M-31 (M-OMe), M-58 and M-102, acyrium ion peaks, m/e 71 from XXb, m/e 57 from XXIb and m/e 43 from XXIIb, were estimated. From these peaks the following fragmentation scheme was proposed.

Chart 4



The NMR spectra of XXb showed twin doublets at 4.65 ppm, and the signal was shifted to 3.00 ppm by deacylation indicating the presence of an O-acyl group at C 4. In addition to the spectral signals of XXIIIb, the presence of a triplet at 0.97 representing C-methyl and an area equivalent to four hydrogens in the 1.2~2.5 ppm region representing the two methylene groups of the acyl chain gave a clear *n*-butyryl group pattern. As with XXb, the twin doublets at 4.65 ppm were found in the spectra of XXIb and XXIIb.

Previously we had reported that the crystalline base, demycarosyl leucomycin A_3 (m.p. 199~202°C) (XIIIa)[8] was obtained by acid hydrolysis of leucomycin A_3(II). Now, after crystallization of XIIIa from a benzene extract of the acid hydrolyzate of II, the crude amorphous base (XIIIb) was obtained from the mother liquor. XIIIb was acetylated with acetic anhydride in pyridine and purified by silicic acid chromatography. Both acetates, XIVa, $C_{36}H_{55}O_{14}N$ (m.p. 196~197°C), and XIVb, $C_{36}H_{55}O_{14}N$ (amorphous), had the same parent peak at m/e 725 by mass spectra. This means that the both acetates are isomers.

The formation of these isomers could be attributed to be due to allylic rearrangement of the hydroxyl group from C_9 to C_{13}. By thorough experiment it was characterized that the amorphous base XIIIb retained the configuration at $C_9 \sim C_{13}$ of leucomycin A_3(II). These studies will be reported in a subsequent report[12].

In order to show that the glycoside linkage of the mycarose moiety was similar to that of leucomycin A_3(II), the latter and component A_4(IV), A_6(VI) and A_8(VIII) were deacylated with barium hydroxide. The presence of desisovaleryl leucomycin A_1(XXIV), $C_{35}H_{59}O_{13}N$, in the hydrolyzates indicates that the mycaroside linkages of these components were identical with that of II.

Thus, the structures of the major compounds described in this report could be written as given on Chart 1. The group having O-acetyl at C_3 on a lactone was named the Ac-group, and that having none was named the Fr-group.

Experimental

NMR spectra at 60 Mc were measured with a Hitachi H-60 spectrometer and those at 100 Mc were measured with a Varian HA-100 spectrometer. Temperatures are uncorrected.

Acetyl leucomycins

Five -hundred mg of each leucomycin component was acetylated with 2 ml of pyridine and 0.5 ml of acetic anhydride. After standing for 20 hours at room temperature the volatile reactants were removed *in vacuo* at 40~50°C. The viscous residue was dissolved in benzene and the solution was chromatographed over silicic acid using benzene-acetone (10 : 1) solvents. The first eluate consisted of acetates of the leucomycins, these being crystallized from CCl_4.

Diacetyl leucomycin A_4(X) (=Triacetyl leucomycin A_5)

Melting point 125~126°C, $[\alpha]_D^{25}$ −80.0° (c 1.0, $CHCl_3$), pKa′ 5.70 (50 % EtOH).

Anal. Calcd. for $C_{45}H_{71}O_{17}N$: C 60.19, H 7.97, N 1.56.
Found : C 60.11, H 7.98, N 1.60.

Diacetyl leucomycin A_6(XI) (=Triacetyl leucomycin A_7)

Melting point 127~129°C, $[\alpha]_D^{25}$ −86.0°C (c 1.50, $CHCl_3$), pKa′ 5.70 (50 % EtOH).

Anal. Calcd. for $C_{44}H_{69}O_{17}N$: C 59.78, H 7.87, N 1.57.
Found : C 59.64, H 7.64, N 1.56.

Diacetyl leucomycin A_8(XII) (=Triacetyl leucomycin A_9)

Melting point 127~130°C, $[\alpha]_D^{25}$ −86.0 (c 1.0, $CHCl_3$), pKa′ 5.70 (50 % EtOH).

Anal. Calcd. for $C_{43}H_{67}O_{17}N$: C 59.37, H 7.76, N 1.61.
Found : C 59.40, H 7.69, N 1.58.

Acid hydrolysis of the leucomycin A_4

Three grams of leucomycin A_4 were dissolved in 60 ml of 0.3 N hydrochloric acid solution and allowed to stand for 20 hours at 5°C. After adjusting to pH 4 with dil. sodium hydroxide, the solution was extracted with chloroform. After removing the solvent, 4-O-*n*-butyryl mycarose (XV) (0.70 g) was obtained as a greenish syrup. The product was used for the following experiment without further purification. The aqueous layer was adjusted to pH 8.0 with dil. sodium hydroxide, extracted with chloroform, dried and evaporated. When the residue was dissolved in benzene, the isomer (0.47 g) having a high melting point precipitated immediately. The precipitate (demycarosyl leucomycin A_3-I) was recrystallized from chloroform.

Melting point 199~203°C (decomp.), $[\alpha]_D^{25}$ −14.1° (c 1.0, $CHCl_3$), pKa′ 7.80 (50 % EtOH)

Anal. Calcd. for $C_{30}H_{49}O_{11}N$: C 60.08, H 8.24, N 2.34.
Found : C 60.20, H 8.19, N 2.30.

The benzene mother liquor was evaporated to dryness *in vacuo* and the residue (demycarosyl leucomycin A_3-II) (XIIIb) (1.145 g) was acetylated without further purification.

Triacetyl demycarosyl leucomycin A_3-I (XIVa)

Two hundred milligrams of XIIIa was dissolved in 2.0 ml of pyridine and 0.5 ml of acetic anhydride. After standing for 20 hours at room temperature, the mixture was treated in the usual manner to yield 0.19 g of XIVa. Recrystallization of XIVa from EtOH-H_2O (12 : 1) gave III as needles, m.p. 195~196°C.

Anal. Calcd. for $C_{36}H_{55}O_{14}N$: C 59.57, H 7.63, N 1.93.
Found : C 60.05, H 7.82, N 1.95.

This compound was identical with triacetyl demycarosyl leucomycin A_3[3] by the NMR, IR and behavior on thin-layer chromatography, m/e $M^+=725$.

Triacetyl demycarosyl leucomycin A_3-II (XIVb)

One gram of demycarosyl leucomycin A_3-II (XIIIb) was acetylated in the same way as was XIIIa and the product was chromatographed on silicic acid. The benzene-acetone (10 : 1) eluate was examined by thin-layer chromatography using Kiesel Gel G. and benzene-acetone (5 : 1). The main eluate (0.7 g) consisted of XIVb. $[\alpha]_D^{25}$ −32.8° (c 5, $CHCl_3$).

Anal. Calcd. for $C_{36}H_{55}O_{14}N$: C 59.57, H 7.64, N 1.93.
Found : C 59.45, H 7.56, N 2.01.

Methyl 4-O-*n*-butyryl mycaroside (XXa) (XXb)

Five hundred milligrams of the crude 4-O-*n*-butyryl mycarose (XV) was dissolved in 30 ml of methanol solution containing 1 % hydrochloric acid. The mixture was refluxed for 2 hours. After removing the methanol *in vacuo* the residue was chromatographed on a 2.0×45 cm column of silicic acid and eluted by benzene-acetone (10 : 1). The α-isomer was eluted first, followed by the β-isomer. These components were confirmed by thin-layer chromatography and the products (450 mg) were distilled *in vacuo*. The properties of these anomers are listed in Table 1, together with those of the methyl-4-O-acyl mycarosides obtained from the other components by a similar method.

Alkaline hydrolysis of α-methyl-4-O-*n*-butyryl mycaroside

Two hundred mg of α-methyl-4-O-butyryl mycaroside was dissolved in 10 ml of 1 N sodium hydroxide and the solution was allowed to stand for 48 hours at room temperature. The hydrolyzate was neutralized with 1 N hydrochloric acid, and submitted to continuous extraction with ether for 6 hours. After drying and evaporating the ether solution the residue was distilled *in vacuo*. The properties of the methyl glycoside (XXIV) (Table 1) coincided with those of the authentic sample obtained from leucomycin A_3 (II). This methyl mycaroside also was obtained from the XXIa and XXIIa by a similar method.

Alkaline hydrolysis of leucomycins

One gram of leucomycin A_3 (II) was dissolved in 50 ml of methanol and the solution was combined with 5.5 ml of saturated aqueous barium hydroxide. After standing for 40 minutes at room temperature the solution was adjusted to pH 7 and extracted with ether. The ether extracts displayed five spots including that from the starting material by thin-layer chromatography [Rf 0.12, 0.20, 0.30, 0.40 and 0.80 (A_3) by using Kiesel Gel G. and methanol-chloroform (1 : 7)]. The alkaline hydrolysis of components IV, VI and VIII in the same manner afforded similar hydrolyzates which also showed five spots of which four had identical Rf values with those of the hydrolyzate obtained from II. These hydrolyzates could not be characterized completely, but the acetate of one component (Rf 0.2 on thin-layer chromatography) which was isolated by silicic acid chromatography using chloroform-methanol (10 : 1) was quite identical with diacetyl leucomycin A_8 (XII).

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