STUDIES ON THE MACROLIDE ANTIBIOTIC YL-704 COMPLEX. II

ISOLATION AND PHYSICOCHEMICAL PROPERTIES OF YL-704 COMPONENTS

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New family of the basic macrolide antibiotics, numbered YL-704, was isolated from the culture broth of *Streptomyces platensis* subsp. *malvinus* MCRL 0388. YL-704 family was constituted of at least thirteen components which were classified into the three types according to their UV absorption pattern; first type (YL-704 W_1 and W_2) with the absorption maximum at 280 nm as carbomycin B, second type (YL-704 A_1, A_0, A_2, A_3, B_1, B_2, B_3 and C_2) with the maiximum at 232 nm as leucomycins, and third type (YL-704 C_1, C_3 and C_4) with no characteristic UV absorption.

In the course of our screening of new antibiotics, a new family of the basic macrolide antibiotic was isolated from the culture broth of *Streptomyces platensis* subsp. *malvinus* MCRL 0388, which is chemotherapeutically active primarily against Gram-positive bacteria. In this paper, the isolation and the physicochemical properties are reported. The taxonomy of the producing strain and the structural elucidation of YL-704 components are reported in detail in the separate papers.[9,10,11]

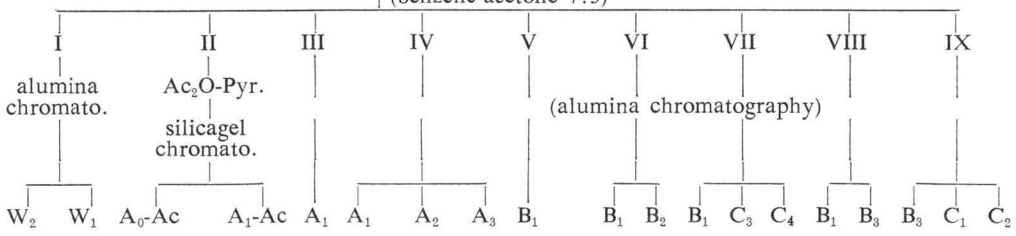
Isolation

As shown in Chart 1, the fermented broth was first adjusted to pH 4.0 and filtered with Celite. The filtrate was extracted twice with ethylacetate at pH 8.0. After concentrating the extract, antibiotics in the ethylacetate layer were transferred into dilute hydrochloric acid of pH 2.0. The acidic aqueous solution was adjusted to pH 8.0 and extracted twice with benzene. The benzene layer was concentrated to dryness to yield the crude powder (15.6 g), which was then dissolved in ethylacetate and rapidly passed through an alumina column for decolorization. The almost colorless powder obtained by the above procedure was then chromatographed on the silica-gel column using the solvent system of benzene-acetone (7:3). Each eluate was monitered on the thin-layer chromatoplate by spraying with 40% H_2SO_4 followed by heating. By the chromatography the antibiotic principles were separated into nine groups. Each group was further purified, where necessary, by the neutral alumina column chromatography to separate the active principle as a pure substance.

YL-704 W_1 and W_2 were separated on an alumina (Woelm, activity I) column with the solvent system of benzene-ethylacetate (3:1). The minor component YL-704 A_0 was not isolated from the large amounts of coexisting YL-704 A_1 by this chromatography, but an acetate com-

Chart 1. Isolation and yields of YL-704 components

Fermented broth (350 mcg/ml)
| filtration with Celite
Filtrate (50 liters)
| adjustment of pH to 8.5
| extraction with ethylacetate (20 liters×2)
Ethylacetate layer
| concentration to 2 liters
| extraction with pH 2.0 water (2 liters×2)
Water layer (4 liters)
| extraction with benzene (4 liters×2) at pH 8 0
| evaporation to dryness
Crude antibiotics (15.6 g)
| decolorization over alumina in ethylacetate
White powder (14.5 g)
| silica gel chromatography
| (benzene-acetone 7:3)



Component	W_2	W_1	A_0—Ac	A_1—Ac	A_1	A_2	A_3
Yield (g)	0.075	0.095	0.025	0.7	3.0	0.2	0.7
Component	B_1	B_2	B_3	C_1	C_2	C_3	C_4
Yield (g)	3.55	0.3	0.8	0.15	0.14	0.011	0.014

Table 1. Physicochemical properties of YL-704 components

Component	YL-704 A_1	YL-704 A_2	YL-704 A_3	YL-704 B_1	YL-704 B_2	YL-704 B_3
Crystal	colorless prism	colorless needle	colorless prism	colorless prism	colorless needle	colorless prism
m.p. (°C)	122~123	193~194	121~122	131~132	185~186	136~137
Formula	$C_{43}H_{71}NO_{15}$	$C_{43}H_{71}NO_{15}$	$C_{42}H_{69}NO_{15}$	$C_{41}H_{67}NO_{15}$	$C_{41}H_{67}NO_{15}$	$C_{40}H_{65}NO_{15}$
M.W. (MS)*	841	841	827	813	813	799
(VP, $CHCl_3$)*	834, 865	822, 854		805, 835	796, 840	
Analysis (%)	Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.
C	61.26 61.36	60.93 61.36	60.81 60.99	60.89 60.52	59.99 60.52	60.20 60.13
H	8.38 8.44	8.36 8.44	8.55 8.41	8.35 8.24	8.15 8.24	8.15 8.20
N	1.88 1.66	1.93 1.66	1.79 1.70	1.71 1.72	1.68 1.72	1.82 1.75
UV λ_{max}^{EtOH} nm	232.5	235	232	232.5	235	232
log ε	4.45	4.22	4.45	4.36	4.36	4.40
IR ν_{max}^{nujol} cm^{-1}	3450 2780 2720 1742 1736 1655 1620 1310 1180 1156 1114 1080 1050 1020 920 845	3485 2780 2725 1740 1735 1720 1655 1630 1321 1182 1161 1100 1082 1050 1032 1005 980	3470 2740 1740 1725 1660 1618 1300 1240 1175 1130 1095 1060 1025 1005 922 845	3450 2780 2725 1745 1738 1670 1640 1312 1290 1180 1160 1118 1080 1050 1020 916 845	3460 2800 2730 1740 1732 1720 1660 1630 1322 1180 1160 1120 1180 1050 1028 1000 918	3460 2720 1738 1720 1660 1635 1305 1238 1168 1122 1090 1055 1020 912 840
$[\alpha]_D^{22}$ (c 1, $CHCl_3$)	−50.2°	−49.0°	−54.0°	−42.1°	−42.0°	−55.0°
pKa′(50% EtOH)	6.90	6.85	6.80	7.00	6.90	6.80

* MS=Mass spectrometry, VP=Vapor pressure osmometry

plex of YL-704 A_0 and A_1 could be separated on silica-gel column chromatography developed with benzene-acetone (5:1) to each acetate. Therefore, the acetate of YL-704 A_0 provided the physicochemical and biological properties.

YL-704 A_1, A_2 and A_3 were successfully isolated on alumina (Woelm, activity I) column by eluting with benzene-ethylacetate (5:2). By chromatography on an alumina (Woelm, activity III) column with benzene: acetone (3:1), YL-704 B_1, B_2, B_3, C_1, C_2, C_3 and C_4 were isolated effectively from each other. The purification procedure was illustrated in Chart 1.

Each component obtained as above was recrystallized from benzene - *n*-hexane as a colorless crystal. The physicochemical properties of every antibiotic components are summarized in Tables 1 and 2.

All components were soluble in methanol, ethanol, propanol, butanol, methylacetate, ethylacetate, chloroform, acetone, diethylether and benzene, sparingly soluble in water, and practically insoluble in *n*-hexane, cylcohexane and petroleum ether. Exceptionally, YL-704 A_2 and B_2 were less soluble in benzene than the other factors.

All antibiotics gave negative erythromycin and carbomycin tests, and negative FEHLING, ferric chloride and ninhydrin tests.[12] On thin-layer chromatography, the antibiotics were visualized as dark violet or purple-colored spots by spraying 40% H_2SO_4 followed by heating,

Table 2. Physicochemical properties of YL-704 components

Component	YL-704 W_2		YL-704 W_1		YL-704 C_1		YL-704 C_2		YL-704 C_3		YL-704 C_4		YL-704 A_0 diacetate	
Crystal	colorless plate		colorless plate		colorless needle		colorless small prism		colorless needle		colorless needle		colorless needle	
m.p.(°C)	101-3		159-161		125-7		116-8		126-7		130-2		114-5	
Formula	$C_{44}H_{71}NO_{15}$		$C_{43}H_{69}NO_{15}$		$C_{41}H_{67}NO_{16}$		$C_{40}H_{65}NO_{15}$		$C_{43}H_{71}NO_{16}$		$C_{42}H_{69}NO_{16}$		$C_{48}H_{77}NO_{17}$	
M.W.(MS)*	853		839		829		799		857		843		939	
Analysis (%)	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
C	61.19	61.95	61.71	61.50	59.45	59.35	60.21	60.08	60.95	60.62	60.17	60.21	61.15	61.39
H	8.25	8.39	8.13	8.22	8.03	8.08	8.20	8.14	8.29	8.38	8.31	8.28	8.21	8.26
N	1.64	1.63	1.70	1.67	1.75	1.69	1.68	1.75	1.72	1.61	1.65	1.63	1.50	1.49
UV λ_{max}^{EtOH} nm	280		280		end		232.5		end		end		232	
log ε	4.37		4.37				4.43						4.47	
IR ν_{max}^{nujol} cm^{-1}	3520	2730	3550	3410	3460	2730	3560	2740	3400	2730	3380	2720	3510	2715
	1740	1680	2750	1748	1740	1660	1740	1725	1720	1660	1742	1720	1735	1660
	1633	1595	1737	1690	1300	1280	1600	1308	1298	1248	1658	1298	1630	1240
	1300	1255	1640	1603	1198	1173	1247	1180	1187	1163	1237	1184	1165	1120
	1200	1175	1300	1200	1090	1065	1135	1094	1132	1083	1163	1132	1055	1030
	1130	1090	1180	1130	1040	980	1060	1025	1052	1033	1083	1050	1000	960
	1060	1025	1090	1065	913	865	927	850	962	918	1034	960	920	
	925	870	1040	1003	842		730		860		915	858		
	845		990	927										
			875											
$[\alpha]_D^{22}$(c 1, $CHCl_3$)			−32°		−69°		−42°							
pKa′(50% EtOH)			6.95		7.00		7.01							

* MS=Mass spectrometry

Table 3. Rf values of YL-704 components on thin-layer chromatography

Component	Rf[a)]	Rf[b)]	Rf[c)]
W_2	0.56	0.92	0.72
W_1	0.37	0.88	0.68
A_0	0.22	0.75	0.51
A_1	0.20	0.72	0.50
A_2		0.60	0.48
A_3		0.53	0.43
B_1		0.58	0.36
B_2		0.45	0.34
B_3		0.38	0.33
C_1		0.30	0.29
C_2		0.43	0.30
C_3		0.41	0.39
C_4		0.29	0.35

a): Alumina-Kieselguhr. Solvents: benzene-acetone (4:1)
b): Alumina-Kieselguhr. Solvents: benzene-acetone (7:3)
c): Silica gel. Solvents: benzene-acetone (2:1)

and their Rf values were shown in Table 3. The antibiotic activities of YL-704 components were given in Table 4 as the minimum inhibitory concentrations against major bacteria by the agar dilution method.

Discussion

The above physicochemical and biological properties of the antibiotics YL-704 suggested the similarity to the 16-membered basic macrolides such as leucomycins,[1] spiramycins,[2] and carbomycins.[3,4]

YL-704 W_1[5] and W_2 exhibited the strong UV maximum at 280 nm of $\alpha, \beta, \gamma, \delta$-unsaturated ketone system as carbomycin B, but were less polar than carbomycin B. In the group showing the UV maximum at 232 nm, YL-704 A_1 and B_1[6] were major products, and YL-704 A_0, A_3, B_3 and C_2 were minor ones, and those suggested the presence of $\alpha,\beta,\gamma,\delta$-

Table 4. MIC (mcg/ml) of YL-704 components by agar dilution method
Medium: Heart infusion agar

Test organisms	W_2	W_1	A_0 acetate	A_1	A_2	A_3	B_1
Staphylococcus aureus FDA 209P	0.78	0.195	0.78	0.195	0.195	0.39	0.78
S. aureus Terashima	1.56	0.39	3.12	0.195	0.195	0.39	0.195
S. aureus Smith	0.78	0.78	6.25	0.39	0.39	0.78	1.56
S. aureus B-20	>100	>100	>100	>100	>100	>100	>100
Bacillus subtilis	0.78	0.195	0.78	0.195	0.195	0.39	0.39
Escherichia coli NIHJ	>100	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae	25	25	100	50	50	100	100
Pseudomonas aeruginosa A_3	100	50	50	100	100	100	100
Proteus vulgaris	>100	>100	>100	>100	>100	>100	>100

Test organisms	B_2	B_3	C_1	C_2	C_3	C_4
S. aureus FDA 209P	0.195	0.78	0.39	0.78	0.39	0.39
S. aureus Terashima	0.195	0.39	0.39	0.78	0.39	0.39
S. aureus Smith	0.78	1.56	0.78	1.56	0.78	0.78
S. aureus B-20	>100	>100	>100	>100	>100	>100
B. subtilis	0.195	0.78	0.39	0.78	0.195	0.195
E. coli NIHJ	>100	>100	>100	>100	>100	>100
K. pneumoniae	50	50	25	50	12.5	12.5
P. aeruginosa A_3	100	100	100	100	100	100
P. vulgaris	>100	>100	>100	>100	>100	>100

unsaturated alcohol system as in leucomycins and spiramycins. YL-704 A_2 and B_2 showed the UV maximum at 235 nm suggesting the existence of the similar $\alpha, \beta, \gamma, \delta$-unsaturated alcohol chromophore. On the TLC, Rf values of YL-704 A_0, A_1 and A_2 were higher than leucomycin A_3.[1b] Rf values of YL-704 A_3 and B_3 were close to leucomycin A_3 and A_6, respectively. Rf values of YL-704 B_1, B_2 and C_2[5] were not identical with those of any components of leucomycins. The each component of YL-704 was readily distinguished from spiramycins by their lipophylic behavior and nitrogen contents. YL-704 C_1[5], C_3 and C_4 indicating the end UV absorption were not identical with any known 16-membered basic macrolide antibiotics.[7]

The yields of the minor components YL-704 A_2 and B_2 were varied in every production. The physicochemical properties and the structure elucidation suggested that YL-704 A_2 and B_2 were the allylic hydroxyl-rearranged compound of YL-704 A_1 and B_1, as in the case of isoleucomycin obtained from leucomycin.[8] Therefore we have assumed them to be artifacts.

The structures of these antibiotics have been elucidated as briefly reported.[5,6,9,10]

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