STUDIES ON THE MACROLIDE ANTIBIOTIC YL-704 COMPLEX.* IV

THE STRUCTURES OF MINOR COMPONENTS

AKIO KINUMAKI, ISAO TAKAMORI, YOICHI SUGAWARA, YOSHIAKI SEKI, MAKOTO SUZUKI and TOMOHARU OKUDA

Microbial Chemistry Research Laboratory,
Tanabe Seiyaku Co., Ltd., Toda, Saitama, Japan

(Received for publication July 18, 1973)

The structures of minor components of new macrolide YL-704, which were produced by *Streptomyces platensis* subsp. *malvinus* MCRL 0388, were elucidated by the physicochemical analyses and the oxidation reaction, and the mass spectrometry of their acetyl derivatives.

The presence of $\alpha, \beta, \gamma, \delta$-dienone and α, β-unsaturated γ, δ-epoxy alcohol chromophore was observed in aglycone portions of minor components, in addition to $\alpha, \beta, \gamma, \delta$-unsaturated alcohol system such as YL-704 A_1 and B_1.

Furtheremore, at the C-3 position in aglycone part, acetyloxy, propionyloxy and butylyloxy functions were determined together with several acyl groups at the end sugar parts.

The eleven minor components of macrolide antibiotic YL-704 were isolated from the culture broth of *Streptomyces platensis* subsp. *malvinus* MCRL 0338.[1] The minor components were studied for the elucidation of their structures[2] by comparison to the major components YL-704 A_1 and B_1.[3,4] They were classified into three groups based on the characteristic UV absorption. The first group with the UV maximum at 280 nm which indicated the $\alpha, \beta, \gamma, \delta$-dienone chromophore contained YL-704 W_1 and W_2. The second group involved YL-704 A_0, A_2, A_3, B_2, B_3 and C_2 with the 232~235 nm maximum absorption of the $\alpha, \beta, \gamma, \delta$-unsaturated alcohol system such as YL-704 A_1, B_1 and leucomycins. The third group which did not present the particular UV absorption included YL-704 C_1, C_3 and C_4. The first group components gave mono acetates, the second and the third produced diacetates by total acetylation.

(1) YL-704 W_1 and W_2:

The analogous IR spectra of YL-704 W_1 and W_2 suggested the functional groups of hydroxy (3550, 3410 cm^{-1}), aldehyde (2750 cm^{-1}), lactone and ester (1748, 1737 cm^{-1}), carbonyl (1690 cm^{-1}) and double bonds (1640, 1603 cm^{-1}). In the PMR of YL-704 W_1 (Fig. 1), the apparent signals were δ ($CDCl_3$) 0.90~1.35 ($7\times CH_3-C$), 2.53 ($-N(CH_3)_2$), 3.57 ($-OCH_3$), 6.05—6.43 (olefinic 4 H) and 9.54 (—CHO).

Besides, the acetyl methyl protones of carbomycin B[5] and the double doublet (9-H) at 4.13 ppm of leucomycin A_3[6] were lacked. YL-704 W_2 showed the same functional groups in PMR to YL-704 W_1.

The both components gave the monoacetates. The mass spectrum of acetyl YL-704 W_1 was shown in Fig. 2, and the spectrum of acetyl YL-704 W_2 was similar except the fragment peaks containing the aglycone portion. The mass fragmentation patterns of them were identical

* Hereafter, we wish to propose the name, platenomycin, in place of the antibiotic YL-704.

with the sugar parts of acetyl carbomycin B and different from the aglycone peaks (AGL^+) of acetyl carbomycin B. The AGL^+ peaks of the acetyl YL-704 W_1 and the acetyl YL-704 W_2 were *m/e* 421 and *m/e* 435, respectively, those were larger by 14 and 28 mass units than the AGL^+(*m/e* 407) of acetyl carbomycin B. On the other hand, AGL^+ —acyloxy fragments of these three compounds were *m/e* 347 equally.

At the aglycone C-3 position, the presence of the propionyloxy function and the butylyloxy function were concluded for YL-704 W_1 and W_2, respectively. Structurally, YL-704 W_1 was identified to be dehydro YL-704 A_1 by MnO_2 oxidation.

(2) YL-704 A_0, A_2, A_3, B_2, B_3 and C_2*:

The IR spectra of YL-704 A_2, A_3, B_2, B_3 and C_2 suggested the presence of same functional groups as YL-704 A_1 and B_1, particularly, in YL-704 A_3, B_3 and C_2 the absorption at 1235 cm^{-1} was observed strongly. YL-704 A_0 was measured as diacetyl derivative which was assumed to be like diacetyl YL-704 A_1.[3,4] YL-704 A_2 and B_2 showed the same UV maximum at 235 nm of log ε 4.34 and log ε 4.30. In mass spectrometry, diacetyl YL-704 A_2 [*m/e* 925 (M^+), 465 (AGL^+), 444 (ADS^+)] and diacetyl YL-704 B_2 [*m/e* 897 (M^+), 465 (AGL^+), 416 (ADS^+)] were suggested the similarity to diacetyl YL-704 A_1 and B_1, respectively. The olefinic protons of of YL-704 A_2 (Fig. 3) and B_2 at 6.0~6.5 ppm in PMR were complex and the same pattern as in the allylic rearranged demycarosyl YL-704 A_1 II.[4]

Those were suggested to be artifacts of YL-704 A_1 and B_1 owing to the uncertain yield of each production. At the treatment

Fig. 1. PMR spectrum of YL-704 W_1

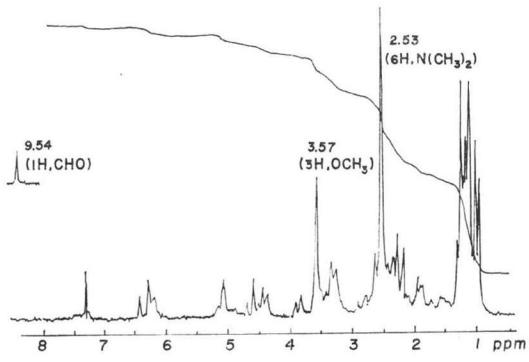
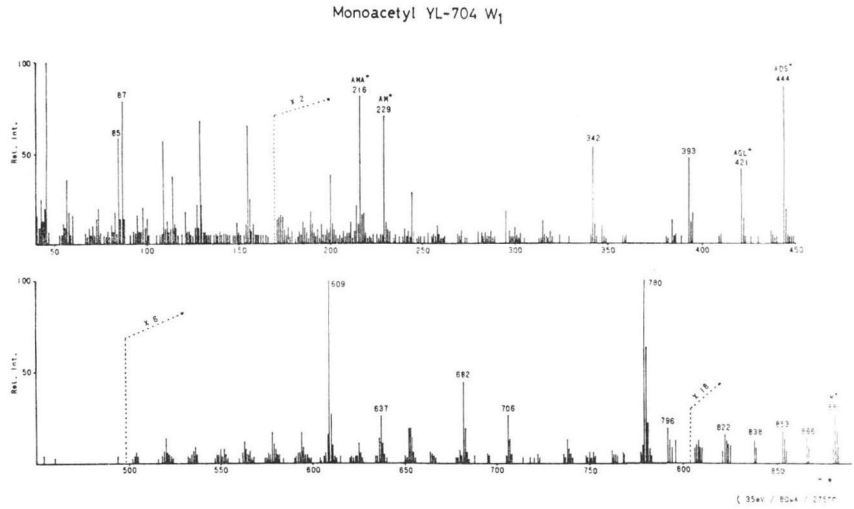


Fig. 2. Mass spectrum of acetyl YL-704 W_1



* YL-704 C_2 was assumed to be same as espinomycin II.[7]

of YL-704 A_1 and B_1 with pH 4 water, the new products were identified to be YL-704 A_2 and B_2 on TLC, respectively. Therefore, YL-704 A_2 and B_2 were decided to be the C-9$\sim$C-13 allylic hydroxy rearranged compounds of YL-704 A_1 and B_1.

The UV maximum of YL-704 A_0, A_3, B_3 and C_2 were observed at 232 nm such as YL-704 A_1. The each structure of their acetates was successfully estimated by the mass spectrometry.

Diacetyl YL-704 A_0 showed *m/e* 939 (M^+), 479 (AGL^+), 419 (AGL^+-CH_3COOH), 331 ($AGL^+-CH_3COOH-CH_3CH_2CH_2COOH$) and 444 ($ADS^+$), of which the AGL^+ was larger by 14 mass units than that of diacetyl YL-704 A_1, and *m/e* 331 and ADS^+ were common to each other. It was concluded that the butylyloxy function was displaced instead of propionyloxy in the aglycone of YL-704 A_1, moreover, the acyl disaccharide was same as YL-704 A_1.

The aglycone peaks of diacetyl YL-704 A_3 and B_3 were both *m/e* 451, and then they were identical to that of diacetyl leucomycin A_3. The ADS^+s were measured to be *m/e* 444 and *m/e* 416, which suggested the same sugar parts as YL-704 A_1 and B_1, respectively. YL-704 A_3 and B_3 were identified to be leucomycin A_3 and A_6,[8] respectively, by m.p., TLC, IR and PMR. YL-704 C_2 showed an acetyl methyl signal at 2.18 ppm in PMR and the aglycone peak of diacetyl YL-704 C_2 was *m/e* 465 such as YL-704 A_1, and ADS^+ showed *m/e* 402. Consequently, the terminal acyl group of sugar part was the acetyl function.

(3) YL-704 C_1, C_3 and C_4*:

The components of this group showed the similar IR spectra to YL-704 A_1, in addition to the characteristic UV of the end absorption. However, YL-704 C_4 was assumed to contain an acetyl group by 1235 cm^{-1} absorption. From the PMR spectrum of YL-704 C_1 (Fig. 4), the olefinic protons were 5.74 ppm (1 H, d, d, J=9, 15 Hz) and 6.14 ppm (1 H, d, d, J=8, 15 Hz) which suggested the partial structure of $-HC-CH=CH-CH-$. YL-704 C_3 and C_4 were analogous to YL-704 C_1 by the physicochemical behaviors.

Fig. 3. PMR spectrum of YL-704 A_2

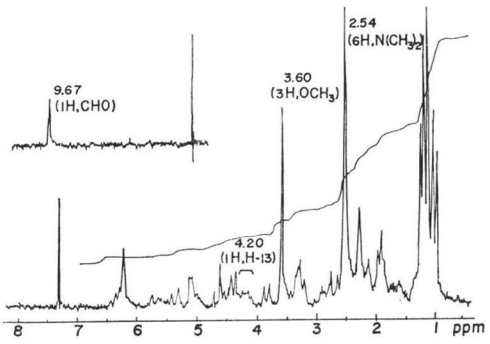
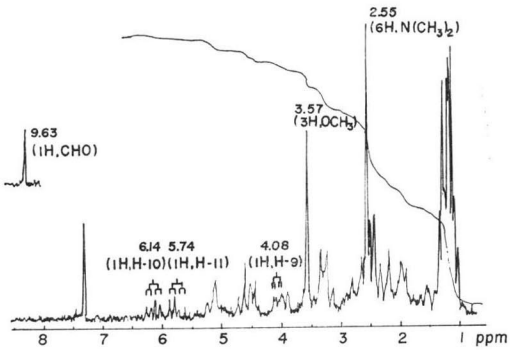


Fig. 4. PMR spectrum of YL-704 C_1



The mass spectra of diacetyl YL-704 C_1 (Fig. 5), C_3 and C_4 were analogous to other YL-704 components, in which AGL^+s were *m/e* 481 for diacetyl YL-704 C_1 and C_3 and *m/e* 467 for diacetyl YL-704 C_4 which were larger by one oxygen than diacetyl YL-704 A_1 and diacetyl leucomycin A_3, respectively. The ADS^+ (*m/e* 416) of diacetyl YL-704 C_1 was identical with diacetyl YL-704 B_1 and those (*m/e* 444) of diacetyl YL-704 C_3 and C_4 suggested the same terminal acyl group as YL-704 A_1. *m/e* 347 ($AGL^+-CH_3COOH-CH_3-COOH$ and $AGL^+-CH_3COOH-CH_3CH_2-$

* YL-704 C_1, C_3 and C_4 were found to be identical with maridomycin III, I and II,[9] respectively.

COOH) was common to the three compounds of this group, which was larger by one oxygen than *m/e* 331 of diacetyl YL-704 A_1 and B_1.

The oxidation of YL-704 C_1 with MnO_2 in $CHCl_3$ gave one dehydro derivative, α,β-unsaturated γ,δ-epoxy ketone with the UV maximum at 239 nm same as carbomycin A.[10] The aglycone chromophore of this group was deduced to be α,β-unsaturated γ,δ-epoxy alcohol.

Fig. 5. Mass spectrum of diacetyl YL-704 C_1

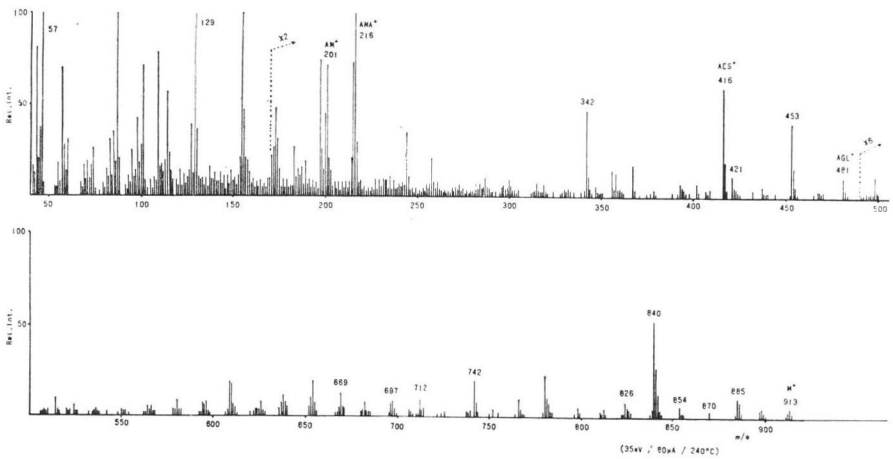
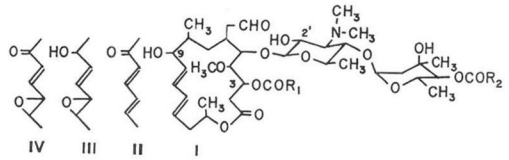


Table 1. Structures of minor components and diagnostic fragment peaks (*m/e*) of their acetates (9, 2′-diacetate/**I**, **III** and 2′-acetate/**II**, **IV**)



Acetate	Chromophore	R_1	R_2	M^+	AGL^+	AGL^+ −9.Ac −R_1COOH	ADS^+	AMA^+	AM^+
YL-704 A_0	I	$-CH_2CH_2CH_3$	$-CH_2CH(CH_3)_2$	939	479	331	444	216	229
YL-704 C_2	I	$-CH_2CH_3$	$-CH_3$	883	465	331	402	216	187
YL-704 A_3	I	$-CH_3$	$-CH_2CH(CH_3)_2$	911	451	331	444	216	229
YL-704 B_3	I	$-CH_3$	$-CH_2CH_3$	883	451	331	416	216	201
YL-704 W_1	II	$-CH_2CH_3$	$-CH_2CH(CH_3)_2$	881	421	347	444	216	229
YL-704 W_2	II	$-CH_2CH_2CH_3$	$-CH_2CH(CH_3)_2$	895	435	347	444	216	229
Carbomycin B	II	$-CH_3$	$-CH_2CH(CH_3)_2$	867	407	347	444	216	229
YL-704 C_1	III	$-CH_2CH_3$	$-CH_2CH_3$	913	481	347	416	216	201
YL-704 C_3	III	$-CH_2CH_3$	$-CH_2CH(CH_3)_2$	941	481	347	444	216	229
YL-704 C_4	III	$-CH_3$	$-CH_2CH(CH_3)_2$	927	467	347	444	216	229
Dehydro YL-704 C_1	IV	$-CH_2CH_3$	$-CH_2CH_3$	869	437	363	416	216	201
Carbomycin A	IV	$-CH_3$	$-CH_2CH(CH_3)_2$	883	423	363	444	216	229

M^+; Molecular ion, AGL^+; Aglycone ion, ADS^+; Acyldisaccharide ion, AMA^+; Acetyl mycaminose ion, AM^+; Acyl mycarose ion.

As the result, the propionyl function was present in YL-704 C_1 and C_3, the acetyl function did in YL-704 C_4, at the C-3 position of the aglycone, respectively.

The structures of all these minor components were elucidated. The diagnostic fragmentation peaks of their acetates were summarized in Table 1.

Experimental

General Method

Melting points are uncorrected. IR spectra were measured with a Hitachi IR 215 and EPI 32 spectrometer. UV spectra were measured with a Hitachi 323 spectrophotometer. NMR-^{1}H spectra were measured at 100 MHz with a JNM 4H-100 and a PS 100 spectrometer. Mass spectra were measured with a Hitachi RMU 7L high resolution mass spectrometer. High resolution mass spectra were measured with a CEC-21 110B high resolution mass spectrometer. Thin-layer chromatography was employed for the detection of the course of reactions and the purity of the products, developed over silica gel GF_{254} (Merck) and alumina (Woelm) with benzene-acetone system. The chromatograms were visualized by heating at 120°C after spraying 40 % H_2SO_4.

Acetates of Minor Components

The acetates of all minor components were prepared with pyridine and acetic anhydride (1 : 1, v/v) at room temperature overnight. After isolating of them by column chromatography on silicic acid (Mallinckrodt) with benzene containing 10 % acetone, the acetates were recrystallized from ethylacetate-*n*-hexane. Their m.p. and elemental analyses were depicted in Table 2.

Oxidation of YL-704 A_1 with MnO_2

YL-704 A_1 (100 mg) was dissolved in 5 ml $CHCl_3$ and added 5 g active MnO_2. After 20-hour stirring at room temperature, the oxidant was filtered off and washed successfully with $CHCl_3$. Chloroform layer was dried and the residue was chromatographed over silica gel with the benzene-acetone (3 : 1) system. Fraction Nos. 8~25 were collected and concentrated to dryness. The product was recrystallized from benzene-*n*-hexane (Yield 73 mg). m.p. 159~160°C.

Anal. calcd. for $C_{43}H_{69}NO_{15}$: C 61.50, H 8.22, N 1.67
Found: C 61.69, H 8.30, N 1.65

This product showed the UV maximum at 280 nm (log ε 4.37) and identical with YL-704 W_1 by IR, NMR and TLC.

Table 2. Melting points and elemental analyses of acetates of minor components

Acetates	m.p.(°C)	Formulae	Calcd. (%)			Found (%)		
			C	H	N	C	H	N
Acetyl YL-704 W_1	188~189	$C_{45}H_{71}NO_{16}$	61.35	8.13	1.59	61.51	8.19	1.59
Acetyl YL-704 W_2	156~157	$C_{46}H_{73}NO_{16}$	61.73	8.22	1.57	61.98	8.40	1.59
Diacetyl YL-704 A_2	115~117	$C_{47}H_{75}NO_{17}$	60.97	8.11	1.51	60.39	8.08	1.50
Diacetyl YL-704 B_2	124~126	$C_{45}H_{71}NO_{17}$	60.20	7.91	1.56	60.22	8.05	1.54
Diacetyl YL-704 A_3	125~126	$C_{46}H_{73}NO_{17}$	60.64	8.08	1.54	60.37	8.33	1.51
Diacetyl YL-704 B_3	127~129	$C_{44}H_{69}NO_{17}$	59.85	7.88	1.59	59.64	7.80	1.55
Diacetyl YL-704 C_2	124~125	$C_{44}H_{69}NO_{17}$	59.85	7·88	1.59	60.02	7.75	1.61
Diacetyl YL-704 A_0	114~115	$C_{48}H_{77}NO_{17}$	61.39	8.26	1.49	61.15	8.21	1.50
Diacetyl YL-704 C_1	102~104	$C_{45}H_{71}NO_{18}$	59.85	7.83	1.53	59.72	7.82	1.54
Diacetyl YL-704 C_3	107~109	$C_{47}H_{75}NO_{18}$	59.99	8.04	1.49	60.37	8.26	1.50
Diacetyl YL-704 C_4	104~106	$C_{46}H_{73}NO_{18}$	59.60	7.94	1.51	59.88	7.84	1.53

Dehydro YL-704 C_1

The mixture of 50 mg YL-704 C_1 and 2 g MnO_2 was stirred in $CHCl_3$ at room temperature for 20 hours. After filtration of MnO_2, chloroform layer was evaporated to yield the crude powder. The crude product was chromatographed over silica gel by benzene-acetone (2 : 1). The corresponding fractions were collected and evaporated. By the crystallization in benzene-*n*-hexane, coloress prisms were obtained (Yield 31 mg). m.p. 128~130°C.

Anal. Calcd. for $C_{41}H_{65}NO_{16}$: C 59.54, H 7.92, N 1.70
Found: C 60.00, H 7.76, N 1.68

UV λ_{max} (EtOH) 239 nm (log ε 4.13).

IR ν_{max} (nujol) 3505, 2720, 1740, 1635, 1165, 1060, 975, 910 cm^{-1}.

Acknowledgement

We wish to express our thanks to Prof. AKIRA TATEMATSU (Meijo University) for providing access to a mass spectrometer, to Prof. KOJI YAMAKAWA (Science University of Tokyo) for the measurement of NMR spectra and to Dr. KEISHI KOTERA and his collaborators of Analytical Center of this company for the instrumental and elemental analyses.

References

1) KINUMAKI, A ; I. TAKAMORI, Y. SUGAWARA, N. NAGAHAMA, M. SUZUKI, Y. EGAWA, M. SAKURAZAWA & T. OKUDA: Studies on the macrolide antibiotic YL-704 complex. II. Isolation and physicochemical properties of YL-704 components. J. Antibiotics 27: 102~106, 1974
2) SUZUKI, M.; I. TAKAMORI, A. KINUMAKI, Y. SUGAWARA & T. OKUDA: The structures of antibiotics YL-704 C_1, C_2 and W_1. J. Antibiotics 24: 904~906, 1971
3) SUZUKI, M.; I. TAKAMORI, A. KINUMAKI, Y. SUGAWARA & T. OKUDA: The structures of antibiotics YL-704 A and B. Tetrahedron Letters 1971: 435-438, 1971
4) KINUMAKI, A.; I. TAKAMORI, Y. SUGAWARA, M. SUZUKI & T. OKUDA: Studies on the macrolide antibiotic YL-704 complex. III. The structures of new macrolide antibiotics YL-704 A_1 and B_1. J. Antibiotics 27: 107~116, 1974
5) a) HOCHSTEIN, F.A. & K. MURAI: Magnamycin B, a second antibiotic from *Streptomyces halstedii*. J. Am. Chem. Soc. 76: 5080~5083, 1954
b) OMURA, S.; A. NAKAGAWA, M. OTANI, T. HATA, H. OGURA & K. FURUHATA: Structure of the spiramycins (foromacidines) and their relationship with the leucomycins and carbomycins (Magnamycins). J. Am. Chem. Soc. 91: 3401~3404, 1969
6) OMURA, S.; M. KATAGIRI, H. OGURA & T. HATA: The chemistry of leucomycins. III. Structure and stereochemistry of leucomycin A_3. Chem. Pharm. Bull. 16: 1181~1186, 1968
7) OMURA, S.; M. KATAGIRI & T. HATA: The chemistry of leucomycins. VI. Structures of leucomycin A_4, A_5, A_6, A_7, A_8 and A_9. J. Antibiotics 21: 272~278, 1968
8) MACHIDA, I.; S. SHIOTSU, K. YOKOTA, S. MAKINO, G. KAWAGUCHI & K. HONDA: Espinomycins. Presented at 178th Meeting of Japan Antibiotics Research Association. March 26, 1971
9) a) MUROI, M.; M. IZAWA & T. KISHI: Structures of maridomycin I, III, IV, V and VI, macrolide antibiotics. Experientia 28: 129~131, 1972
b) MUROI, M.; M. IZAWA, H. ONO, E. HIGASHIDE & T KISHI: Isolation of maridomycins and structure of maridomycin. II. Experientia 28: 501~502, 1972
10) a) WOODWARD, R.B.: Struktur und Biogeneses der Macrolide, eine neue Klasse von Naturstoffen. Angew. Chem. 69: 50~58, 1957
b) WOODWARD, R.B.; L.S. WEILER & P.C. DUTTA: The structure of magnamycin. J. Am. Chem. Soc. 87: 4662~4663, 1965