STUDIES ON THE ISOTETRACENONE ANTIBIOTICS

II. KERRIAMYCINS A, B AND C, NEW ANTITUMOR ANTIBIOTICS

Sir:

During the course of our screening program for new antitumor antibiotics, we have recently isolated three active substances which were named kerriamycins A, B and C, respectively, from the culture broth of a streptomycete. These antibiotics inhibit the growth of Gram-positive bacteria and prolong the survival periods of mice bearing Ehrlich ascites carcinoma. Each

Fig. 1. Structures of kerriamycins.

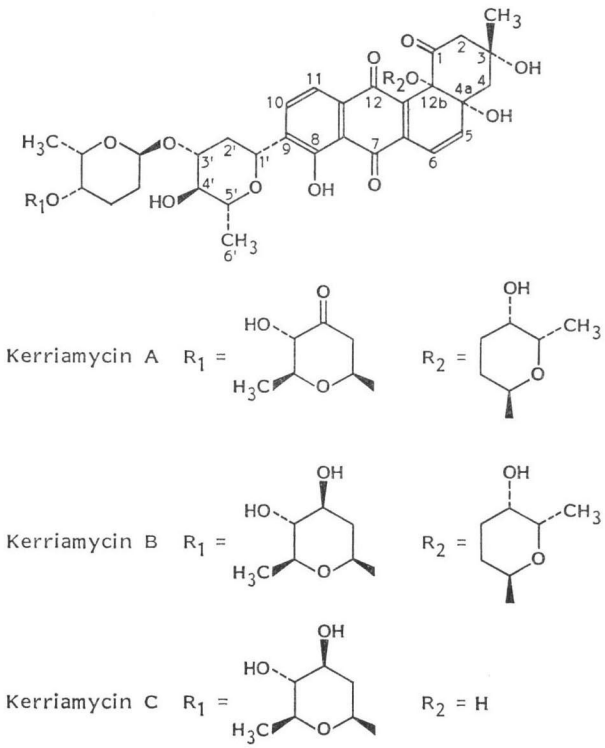

Table 1. Physico-chemical properties of kerriamycins.

	Kerriamycin A	Kerriamycin B	Kerriamycin C
Nature	Yellow powder	Yellow powder	Yellow powder
MP (dec)	177~182°C	188~193°C	176~181°C
$[\alpha]_D^{21}$ (c 0.1, MeOH)	+55°	+39°	+37°
FAB mass (m/z) $(M+Na)^+$	865	867	753
Analysis (calcd)			
C	61.05 (61.27)	61.12 (61.13)	60.84 (60.81)
H	6.52 (6.46)	6.84 (6.68)	6.61 (6.35)
O	32.43 (32.27)	32.04 (32.19)	32.55 (32.84)
Formula	$C_{43}H_{54}O_{17}$	$C_{43}H_{56}O_{17}$	$C_{37}H_{46}O_{15}$
UV λ_{max} nm ($E_{1cm}^{1\%}$)			
MeOH	220 (349), 318 (61), 423 (70)	220 (345), 319 (58), 422 (70)	219 (369), 316 (70), 421 (78)
0.01 N NaOH - MeOH	227 (355), 323 (108), 400 (29), 578 (65)	228 (356), 323 (105), 403 (29), 580 (63)	227 (373), 318 (117), 390 (37), 553 (63)
IR KBr (cm^{-1})	3430, 1725, 1655, 1637	3440, 1725, 1655, 1637	3400, 1720, 1655, 1638

Table 2. ^{13}C Chemical shift assignments of kerriamycins and aquayamycin.

	Kerriamycin A ($CDCl_3$) ppm	Kerriamycin B (CD_3OD) ppm	Kerriamycin C (CD_3OD) ppm	Aquayamycin (CD_3OD) ppm
1	204.9	204.0	206.1	206.1
2	53.9	54.8	53.2	53.1
3	75.3	77.0	77.5*	77.4*
3-CH_3	29.9	30.1	30.3	30.3
4	43.3	44.5	44.8	44.7
4a	81.6	82.6	81.9	81.8
5	144.5	146.0	145.8	145.8
6	116.4	117.6	117.9	118.0
6a	136.9	138.2	138.7	138.8
7	187.2	189.0	189.0	188.8
7a	113.5	115.1	115.1	114.8
8	157.4	158.2	158.2	158.0
9	138.1	138.7	139.5	139.4
10	133.4	134.1	134.0	133.8
11	119.7	120.0	119.7	119.8
11a	129.8	131.9	131.8	131.4
12	181.6	183.4	182.9	182.7
12a	138.5	141.1	140.0	139.7
12b	80.2	82.4	77.8*	78.4*
1′	71.0	72.2	72.2	72.2
2′	37.5	37.9	37.8	40.9
3′	81.3	77.9	77.5	73.4
4′	75.9	76.7	76.7	78.6
5′	76.0	77.6	77.6	77.4
6′	18.5	19.0	19.0	18.8
Rhodinose 1				
1	96.9	95.2	95.1	
2	25.1	25.7	25.7	
3	24.5	25.5	25.5	
4	76.4	77.5	77.5	
5	67.1	67.8	67.7	
6	17.1	17.5	17.5	
Rhodinose 2				
1	94.4	95.4		
2	23.1	24.3		
3	25.4	26.5		
4	66.8	67.8		
5	67.0	68.1		
6	16.6	17.0		
Kerriose (Olivose)				
1	101.6	102.6	102.6	
2	46.9	40.7	40.7	
3	201.1	72.2	72.2	
4	78.0	78.3	78.3	
5	72.6	73.1	73.1	
6	18.8	18.5	18.5	

* Interchangeable.

kerriamycin belongs to the isotetracenone antibiotic group[1], members of which contain the molecule of aquayamycin[2] together with two or three hexoses. The structures of the kerriamycins are depicted in Fig. 1.

The kerriamycin producing organism which was identified as *Streptomyces violaceolatus* was cultivated on a rotary shaker at 27°C in 500-ml

Table 3. Antitumor activities of kerriamycins A and B against Ehrlich mouse ascites carcinoma.

	Dose (mg/kg/day)	T/C* (%)	50 days survivor**
Kerriamycin A	50	Toxic	0/5
	25	70	0/5
	12.5	208	0/5
	6.25	205	0/6
	3.13	>196	1/6
	1.56	136	0/6
Kerriamycin B	100	Toxic	0/5
	50	152	0/5
	25	133	0/5
	12.5	208	0/5

Treatment schedule: day 1, 5 (ip).

* The ratio of mean survival time of the treated group divided by that of the control group.

** The number of surviving mice on day 50 among the tested mice.

Erlenmeyer flasks containing 100 ml of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4%.

It is noticeable that the time course of kerriamycin A production differs from those of B and C, suggesting the biosynthetic sequence of these antibiotics.

The 4-day-cultured broth (1 liter) was filtered and the filtrate was extracted with EtOAc. The organic layer was concentrated *in vacuo* and subjected to silica gel column chromatography. The active fraction was eluted with $CHCl_3$-MeOH (20: 1) and evaporated *in vacuo* to give crude kerriamycin A. This material was applied to a Toyopearl HW40F column, which was developed with MeOH. The active eluate was concentrated to dryness to give a yellow powder (160 mg) of kerriamycin A in pure form.

The 1-day-cultured broth (1 liter) was used for the isolation of kerriamycins B and C. After extraction of the broth filtrate with EtOAc, the solvent layer was concentrated *in vacuo* and subjected to silica gel column chromatography. The active fraction was eluted with $CHCl_3$-MeOH (10: 1) and evaporated *in vacuo* to give a mixture of kerriamycins B and C. This mixture was further purified by Toyopearl HW40F column chromatography. Development of the column with MeOH gave two yellow bands, which were separately collected and concentrated to dryness to give yellow powders of kerriamycin B (300 mg) and kerriamycin C (40 mg), respectively.

The physico-chemical properties of kerriamycins A, B and C are as shown in Table 1.

Hydrolysis of the kerriamycins in 2 N HCl for 2 hours at room temp gave aquayamycin, which was extracted with EtOAc and identified by direct comparison with an authentic sample. The remaining sugar moieties of kerriamycins were determined by NMR analysis and degradation studies. The locations of these sugars were elucidated by NOE enhancements on irradiation of the anomeric protons.

Kerriamycin A contains two units of L-rhodinose[3] and one unit of a 2,6-dideoxy-*erythro*-hexopyran-3-ulose which is named kerriose. As far as we know, kerriose is a new sugar found for the first time from a natural source. Kerriamycin B comprises two units of L-rhodinose and one unit of D-olivose[4], while

Table 4. Antimicrobial spectra of kerriamycins (MIC, μg/ml).

Organism	Kerriamycin A	Kerriamycin B	Kerriamycin C
Staphylococcus aureus FDA 209P	12.5	12.5	12.5
Bacillus subtilis ATCC 6633	12.5	12.5	25.0
B. cereus IAM 1729	12.5	12.5	12.5
Micrococcus luteus ATCC 9341	6.25	6.25	6.25
Escherichia coli NIHJ JC-2	>100	>100	>100
Klebsiella pneumoniae PCI-602	>100	>100	>100
Salmonella typhimurium IID 971	>100	>100	>100
Pseudomonas aeruginosa NCTC 10490	>100	>100	>100
Saccharomyces cerevisiae ATCC 9763	>100	>100	>100
Candida albicans No. Yu 1200	>100	>100	>100
Aspergillus fumigatus IFO 4400	>100	>100	>100
Penicillium chrysogenum ATCC 10002	>100	>100	>100
Trichophyton mentagrophytes	>100	>100	>100

kerriamycin C contains one unit each of L-rhodinose and D-olivose. The ^{13}C NMR assignments of the kerriamycins and aquayamycin are summarized in Table 2. The structural studies will be reported in due course.

Table 3 shows the effects of kerriamycins A and B on Ehrlich mouse ascites carcinoma. The intraperitoneal injections of kerriamycins on days 1 and 5 caused prolongation of the life spans of treated mice. The antimicrobial activities of kerriamycins are shown in Table 4.

Further studies on the biological activities of kerriamycins are in progress and will be published in the future.

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