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STRUCTURE OF KIKUMYCIN A AND B

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Kikumycin A and B are basic antiviral antibiotics isolated from the culture filtrate of Streptomyces phaeochromogenes R-719[1]. After their precise physicochemical data, especially the mass spectral information have become available, the empirical formula previously proposed for Kikumycin A (Ia), ($C_{24}H_{35}O_9N_{11}$) and for B (Ib), ($C_{13}H_{17}O_2N_7$), should be corrected as follows. Ia-sulfate : colorless fine needles, mp. 285°C (dec), $[\alpha]_D^{25}$ +12.8° (c=1, 1N-HCl), pKa' 9.70 and >12.0, IR : 3400, 1660, 1580, 1240, 1090 (cm^{-1} in Nujol), UV : 238nm (log 4.17) and 322.5nm (4.51) in 0.1N-HCl, 357nm (4.50) in 0.1N-NaOH, Anal.*Calcd. for $C_{13}H_{17}O_2N_7 \cdot H_2SO_4 \cdot H_2O$: C, 37.26; H, 4.57; N, 23.40; S, 7.65%, Found : C, 37.89; H, 4.80; N, 23.39; S, 7.62%. Ib-hydrochloride : mp. 220-3°C, $[\alpha]_D^{25}$ +14° (c=1, H_2O), pKa' 9.75 and >12.0, IR : 3400, 1660, 1600, 1230, 1060, UV : 242nm (4.02) and 326nm (4.29) in 0.1N-HCl, 360nm (4.24) in 0.1N-NaOH, Anal.*Calcd. for $C_{14}H_{19}O_2N_7 \cdot 2HCl \cdot 3/2H_2O$: C, 40.32; H, 5.80; N, 23.51; Cl, 17.01 %, Found : C, 40.42; H, 5.43; N, 22.82; Cl, 17.72%.

Ia and Ib reacted positively with modified Ehrlich's and Grote's reagents. From the above physicochemical properties, it is clear that both Ia and Ib belong to the group of "pyrrole-amidine" antiviral antibiotics such as Netropsin[2], Distamycin A[3] and Anthelvencins[4]. However, the chromophore of Ia and Ib dose not seem to be the isolated 4-amino-2-carboxy-pyrrole system, the common chromophore of the above antibiotics, judging from the longer UV maxima and the bathochromic shifts in alkaline solution.

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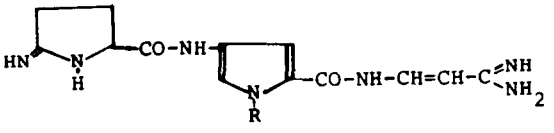
*Since accurate analytical data are hardly obtainable in these types of antibiotics, the mass-spectrometric information played important roll in these cases.

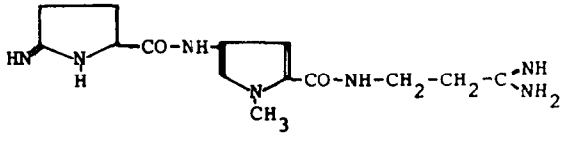
The pmr spectra[5] of Ia and Ib exhibited imino-pyrrolidine ring methylene and methine protons (Ia : δ2.56, 3.05, 4.63; Ib : δ2.55, 3.05, 4.67), pyrrole aromatic protons (Ia : δ6.92, 7.30; Ib : δ6.97, 7.24), and AB-type olefinic protons (Ia : δ5.62, 7.85; Ib : δ5.62, 7.88) coupled with a large coupling constant (15Hz) suggesting the trans configration. Moreover, the following pmr data revealed that the structures of Ia and Ib differed by one methyl group. The spectrum of Ia indicated a pyrrole -NH proton at δ11.85 (1H, broad), while Ib pyrrole $-NCH_3$ protons at δ3.67 (3H, S).

Catalytic hydrogenation of Ib afforded dihydrokikumycin B (II). II-sulfate : mp. 243-4°C, $[\alpha]_D^{25}$+15° (c=1, H_2O), pKa' 9.80 and >12.0, IR : 3320, 1705, 1660, 1600, 1110(cm^{-1} in Nujol), UV : 237nm (4.02) and 278nm (3.92) in H_2O, Anal. Calcd.for $C_{14}H_{21}O_2N_7 \cdot H_2SO_4 \cdot H_2O$: C, 38.65; H, 5.79; N, 22.54; S, 7.37 %, Found : C, 38.23; H, 5.86; N, 22.06; S, 7.36%.

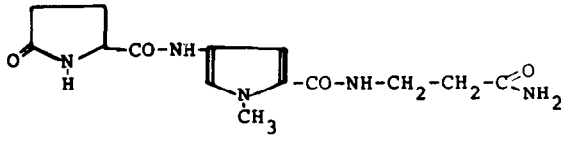
Both UV maxima of II showed no bathochromic shift in alkaline solution as in the case of antibiotics possessing the isolated 4-amino-2-carboxy-pyrrole chromophore. Comparison of UV data between Ib and II suggests that the double bond located in Ib participates in the chromophoric pyrrole-π-electron system and the double bond is saturated in II. Actually, in the pmr spectrum of II, new methylene protons (δ2.67 and 3.73) were observed instead of two olefinic protons.



Ia (R=H) : kikumycin A
Ib ($R=CH_3$) : kikumycin B



II : dihydrokikumycin B



III : dihydrokikumycin B amide

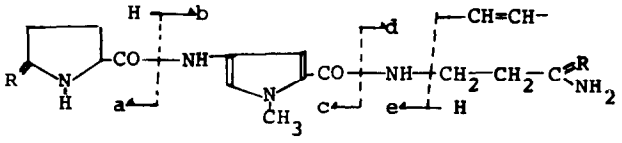
By acid hydrolysis, Ia and Ib afforded only glutamic acid which was ascribed to the 2-carboxy-5-imino pyrrolidine moiety, while II gave glutamic acid β-alanine and minor ammounts of 2-amino-ethylamidine. Isolation of the latter

two hydrolysates supported the presence of a 2-amino-ethylamidine moiety in II, which was lacking in Ia and Ib[6]. It was apparent that the 2-amino-ethylamidine moiety of II was derived from the terminal amidine part of Ib by catalytic hydrogenation.

Mild alkaline hydrolysis of II afforded a neutral compound, dihydrokikumycin B amide (III) : mp. 203-6°C, $[\alpha]_D^{25}$ +11°(c=1, H_2O), IR : 3200, 1675, 1650, 1595, 1405 (cm^{-1} in Nujol), UV : 237nm (3.89) and 278nm (3.75), Anal. Calcd. for $C_{14}H_{19}O_4N_5$: C, 52.38; H, 5.97; N, 21.82%, Found : C, 52.19; H, 6.02; N, 21.63%, mass, m/e 321 (M^+). In the pmr spectrum of III, all the amidine protons of II (δ8.30, 3H and 9.20, 4H) disappeared and amide protons (δ7.90, 2H broad and 9.50, 1H broad) were observed. These data indicated that both amidine groups of II were changed to the amide functions in III.

The amino acid sequence and structure determinations of kikumycins were performed mainly by application of mass spectrometry. In the mass spectra of Ib-hydrochloride and II-hydrochloride, molecular ions were not observed, but M^+ -NH_3 ions (Ib : m/e 300; II : m/e 302) were clearly recognized. In contrast with these amidine salts, the sufficient volatility of III afforded the molecular ion (m/e 321), M^+-H_2O (m/e 303) and fragment peaks useful for deciding the molecular formula and amino acid sequence. The fragment ions that were important for the determination of the amino acid sequence were those derived from CO-NH bond cleavage as summerized in Table I.

Table I. Sequencial fragments of kikumycin B derivatives.



	a	b	c	d	e
Ib	111	190*	—	—	249
II	111	192*	233	69*	249
III	112	210	234	87	250

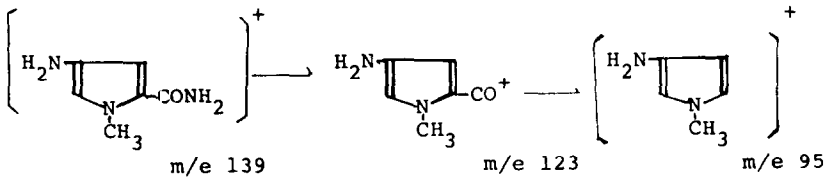
*Those fragments were produced from successive NH_3 elimination in b or d cleavage.

Fragment ions from c or d cleavage in II and III, namely the peaks listed in Table I and m/e 139, 123 and 95 (Scheme I), were observed in low intensities

in the mass spectrum of Ib.

One of the reasons for this seems to be the stabilization of the molecule due to the enolization from -CO-NH- to -C(OH)=N- in the 2-carboxamide structure by the influence of the adjacent olefine function.

A bathochromic shift observed in UV spectra of Ia and Ib in alkaline solution may be explained similarly, and these details will be published elsewhere.



Scheme 1.

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4) G. W. Probst, M. M. Hoehn and B. L. Woods : Antimicrobial Ageants and Chemotherapy 1965,789.

5) Pmr were measured by 60 MHz with TMS as an internal standard. (Solvent : D_2O in 0-8 ppm. range, d_6-DMSO in 7-14 ppm and -NH ranges.)

6) 2-Amino-ethylamidine was identified as a bright-yellow ninhydrin spot on p. c. g. of the aqueous or methanolic hydrolysate of II. S. Nakamura : Chem. Pharm. Bull. (Tokyo) 9, 641 (1961).