A NEW ANTIBIOTIC XK-62-2. III

THE STRUCTURE OF XK-62-2, A NEW GENTAMICIN C COMPLEX ANTIBIOTIC

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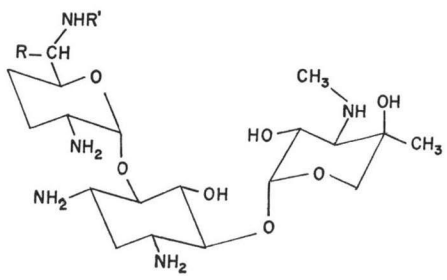
The structure of XK-62-2 has been firmly established to be 6′-*N*-methylgentamicin C_{1a} (**3**) by application of spectroscopic methods in conjunction with chemical degradation. The data obtained in every case are completely consistent with the proposed structure.

The gentamicin C complex[1] has been reported to consist of 3 antibiotics[2,3] designated C_1, C_2 and C_{1a} (**1**, **2** and **4**)[4]. These compounds differ only in the methyl substitution pattern of the hexo- or heptopyranoses attached to the 4-position of 2-deoxystreptamine—purpurosamines A, B and C[5]. We now wish to report the structure of XK-62-2[6,7] which is 6′-*N*-methylgentamicin C_{1a} (**3**) the missing member of the gentamicin C complex. This compound completes the identification of all mono- and di-methyl substituted purpurosamine compounds.



	R	R′	
1	CH_3	CH_3	Gentamicin C_1
2	CH_3	H	Gentamicin C_2
3	H	CH_3	XK-62-2
4	H	H	Gentamicin C_{1a}

When the acid hydrolysates of XK-62-2 and gentamicins C_1, C_2 and C_{1a} were compared, XK-62-2 was found to contain 2-deoxystreptamine and a purpurosamine which differed in Rf from purpurosamines A, B and C as described by COOPER *et al.*[5] (*cf.* Table 1). The *N*-pertrifluoroacetyl-*O*-pertrimethylsilyl derivative of XK-62-2 was also found to have a different retention time on

Table 1. T.L.C. Mobilities of purpurosamines

Antibiotic	Purpurosamine	Rf-system 1*	Rf-system 2**
C_1	A	0.625	0.208
C_2	B	0.575	0.208
C_{1a}	C	0.475	0.146
XK-62-2	—	0.550	0.167

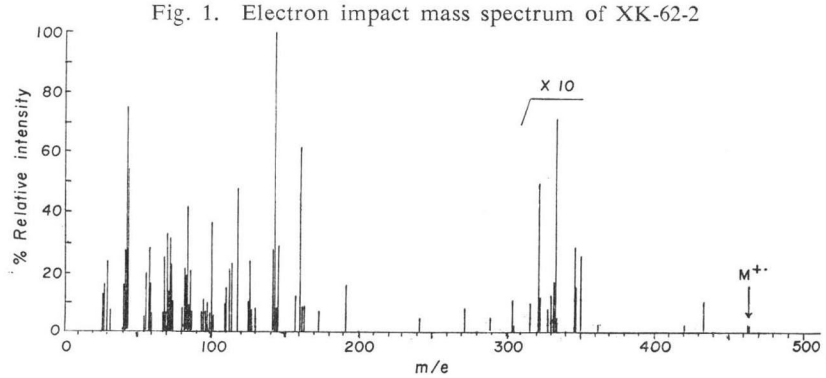
* Propanol-pyridine-acetic acid-water (15:10:3:12)
** Pyridine-ethylacetate-acetic acid-water (5:6:1:3). Measured on cellulose

Table 2. T.L.C. Mobilities of gentamicin C complex

Antibiotic	Rf*
C_{1a}	0.45
C_2	0.51
XK-62-2	0.54
C_1	0.59

* Methanol - chloroform - ammonia (1 : 1 : 1, lower phase). Measured on Quanta Gram silica gel and detected with ninhydrin.

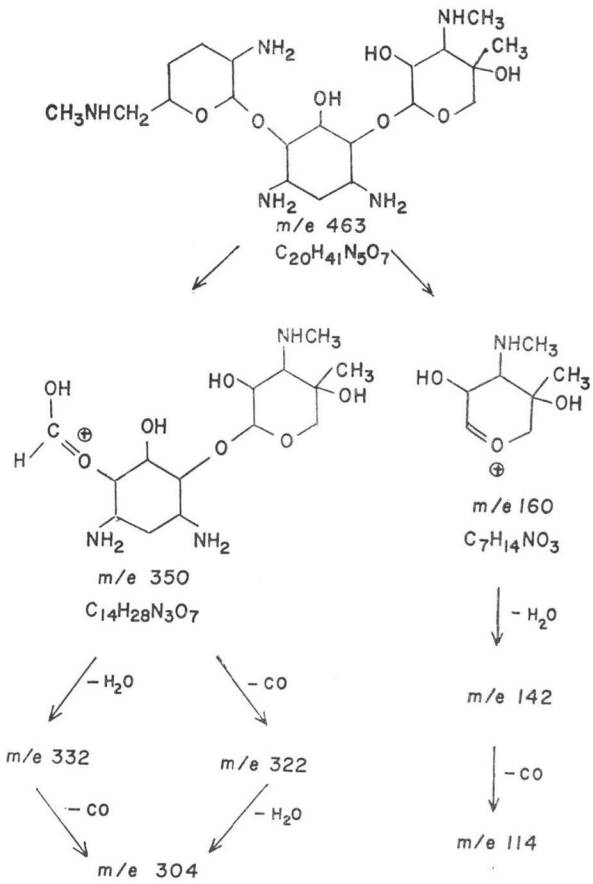
Fig. 1. Electron impact mass spectrum of XK-62-2



glc than the corresponding derivative of the known gentamicins.* XK-62-2 has an Rf slower than C_1 but faster than C_2 when chromatographed on silica gel developed with MeOH-$CHCl_3$-NH_4OH (1:1:1, lower phase) (*cf.* Table 2). Therefore the new gentamicin was postulated to be slightly more basic than C_2 but less basic than C_1. Since the presence of a methyl group on an amino substituent should increase its basisity, it was suggested that the new gentamicin was 6′-*N*-methylgentamicin C_{1a}.

High resolution mass spectra were obtained for XK-62-2 and the three gentamicin C complex reference compounds[8]. The underivatized compounds gave good spectra with prominent $M^{+\cdot}$ and $(M+1)^+$ ions. The mass spectrum of XK-62-2 is shown in Fig. 1, and important fragment ions are shown in Chart 1. Empirical formulae for $M^{+\cdot}$ and significant fragment ions were obtained by high-resolution data acquisition or peak matching. XK-62-2 gave an *m/e* 463 ($C_{20}H_{41}N_5O_7$) molecular ion indicating that it was isomeric with gentamicin C_2. A peak at *m/e* 160 ($C_7H_{14}NO_3$) arising from garosamine was present in all four spectra. An *m/e* 304 ($C_{13}H_{26}N_3O_5$) fragment resulting from cleavage of the purpurosamine moiety was also present in each case suggesting that XK-62-2 and the gentamicins had identical pseudodisaccharide fragments. Compounds **1**, **2** and **3** all have a fragment at *m/e* 420 ($C_{18}H_{36}N_4O_7$) which corresponds to losses of 57 (C_3H_7N), 43 (C_2H_5N) and 43 (C_2H_5N) from the respective

Chart 1. Mass spectral fragmentation of XK-62-2

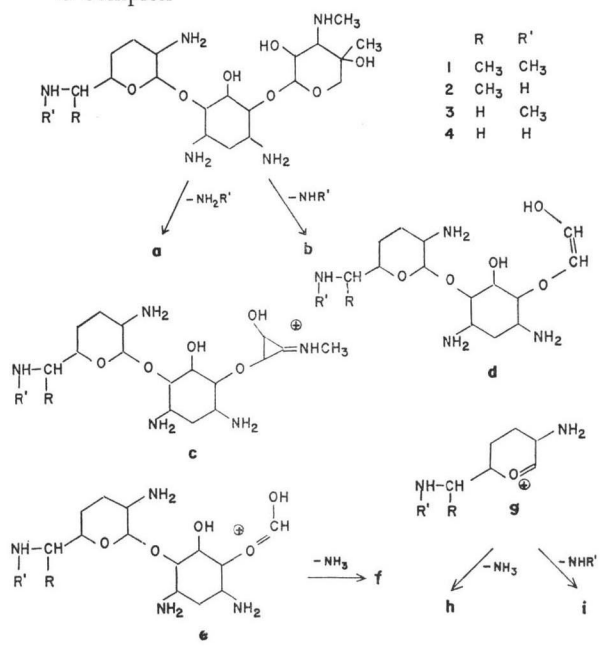


* R. J. MAURITZ, Abbott Laboratories, manuscript in preparation.

molecular ions. The ions at *m/e* 463, 420, 304 and 160 together, localize the differences between the various compounds to the purpurosamine ring side chain.

Numerous purpurosamine containing fragments mirror this difference in side-chain composition (**a**~**i**, Chart 2 and Table 3). Most significant in the cases of XK-62-2 and gentamicin C_1 is the loss of CH_3NH which suggests that both compounds have 6′-*N*-methyl substitution.

Chart 2. Mass spectral fragmentation of gentamicin C complex



Additional evidence that firmly establishes the position of methylation was afforded by the ^{13}C-nmr spectrum of XK-62-2 compared with published data[9] of the other gentamicin C complex substances (Table 4). The chemical shifts of C-5′ and C-6′ respectively show characteristic upfield γ- and downfield β-shifts indicative of 6′-methylation of gentamicin C_{1a}. The remaining carbon resonances are virtually unchanged from the reference.

The ^1H-nmr spectra of XK-62-2 and gentamicin C_{1a} sulfate salts (Fig. 2) reveal two anomeric proton resonances indicating that the compounds are pseudotrisaccharides. The

Table 3. Mass spectral fragmentation of gentamicin C complex. Mass of prominent ions*

Compound	Ions								
	a	b	c	d	e	f	g	h	i
1	—	447	402	360	347	330	157	140	126
2	446	—	388	346	333	316	143	126	—
3	—	433	388	346	333	316	143	126	112
4	432	—	374	332	319	302	129	112	—

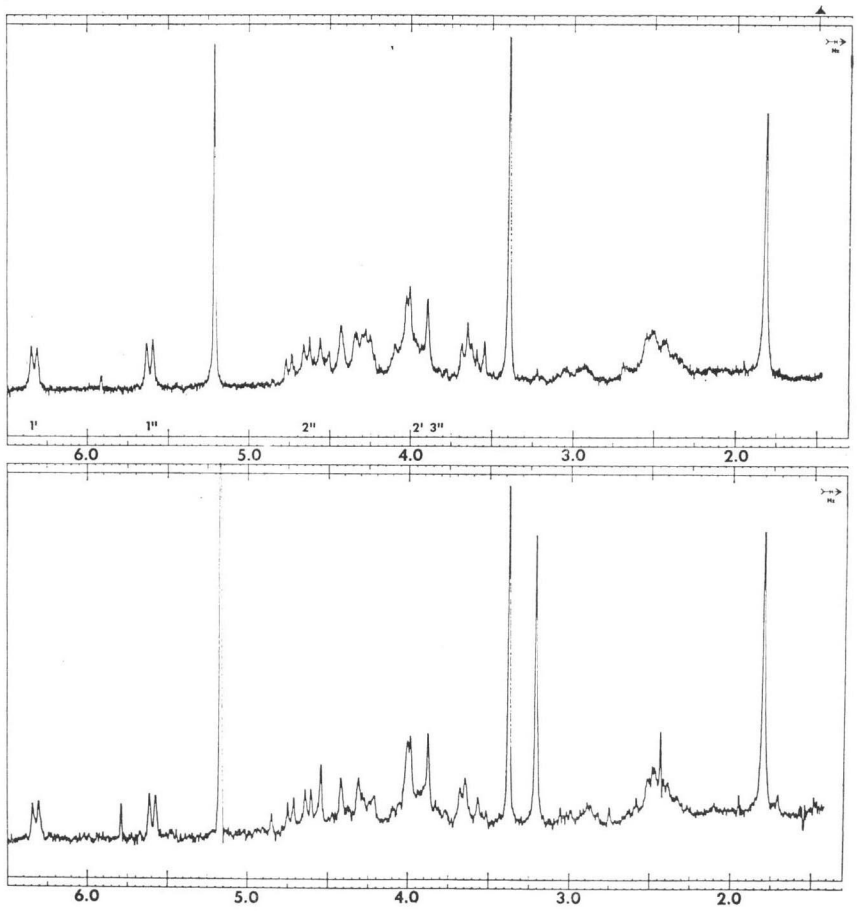
* Identified in Chart 2

Table 4. ^{13}C-Nmr data of XK-62-2 (**3**) and gentamicin C_{1a} (4)

	3	4		3	4		3	4
C-1	51.5	51.7	C-1′	101.2	102.2	C-1″	101.2	101.3
C-2	36.5	36.7	C-2′	50.5	51.0	C-2″	70.1	70.2
C-3	50.3	50.6	C-3′	26.5	27.1	C-3″	64.2	64.4
C-4	87.7	88.3	C-4′	28.5	28.5	C-4″	73.1	73.3
C-5	75.4	75.4	C-5′	68.5	71.5	C-5″	68.1	68.7
C-6	86.9	87.8	C-6′	55.1	46.0	3″-NCH_3	37.7	38.0
			6′-NCH_3	35.3	—	4″-CCH_3	22.5	23.0

5.62 ppm anomeric resonance of gentamicin C_{1a} sulfate is attributable to garosamine on the basis of spin decoupling experiments which locate the resonance of H-2″ at 4.70 ppm as a doublet of doublets ($J_{1'',2''}=3.5$ Hz, $J_{2'',3''}=11.0$ Hz) and the resonance of H-3″ as a simple

Fig. 2. ^{1}H-Nmr spectrum of gentamicin C_{1a} sulfate (top) and XK-62-2 sulfate (bottom) in D_2O solution



doublet at 3.95 ppm. The lack of a second coupling for H-3″ indicates that the 4″-position is tertiary as is expected in garosamine. A protonated *N*-methyl singlet at 3.38 ppm and a tertiary *C*-methyl singlet at 1.80 ppm are also attributable to garosamine present in both compounds.

The patterns of the ring proton resonances between 2.0 and 5.0 ppm are unchanged indicating that no change in carbon skeletons has occurred. The only significant difference between the two spectra is the presence of an additional protonated *N*-methyl singlet at 3.21 ppm in the spectrum of XK-62-2 sulfate. This singlet can be attributed to a 6′-*N*-methyl substituent of the purpurosamine. The absence of an upfield *C*-methyl doublet in the spectrum of XK-62-2 sulfate indicates that this compound, like gentamicin C_{1a}, does not contain the 6′-*C*-methyl purpurosamine found in gentamicin C_1 or C_2.

Comment

It is appropriate at this point to comment on the probable relationship between XK-62-2 and the material isolated by KERSHNER and designated gentamicin C_{2a}[10]. Critical to the proof of structure offered by KERSHNER are mass spectral and nmr spectral analyses. The published interpretation of the nmr spectrum of gentamicin C_{2a} includes the controversial report that the resonance of the 6′-*N*-methyl group appears as a doublet due to coupling with the NH proton which was possible because of slow exchange in D_2O solution. Examination of the published spectrum supports an alternate explanation that the doubling of peaks is a consequence of the presence of substantial amounts of other compounds—most likely gentamicin C_1. The published gentamine C_{2a} spectrum clearly reveals a similar lack of purity.

A stronger basis for KERSHNER's conclusion is his mass spectral data[10,11] which are essentially identical to that which we report. Particularly noteworthy is the loss of CH_3NH from the parent ion as confirmed by metastable defocussing. It remains possible however, in light of the close structural similarities of the components of the gentamicin C complex, that substantial amounts of contaminant could go undetected by mass spectrometry.

In summary although it appears likely that a portion of the mixture isolated and studied by KERSHNER is identical with our XK-62-2 the proof based on his published data is not unambiguous.

Experimental

Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 50 eV and 170°C using the direct insertion probe. ^{1}H-nmr spectra were measured on a Varian Associates HA-100 spectrometer in D_2O solution. Chemical shifts are reported in ppm downfield from external TMS contained in the inner tube of a Wilmad Co-axial cell assembly. ^{13}C-Nmr were measured on a Varian Associates XL-100 spectrometer in D_2O solution. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. The authors are indebted to Dr. D. HILLENBRAND, University of Wisconsin for the ^{13}C-nmr data.

Acid Hydrolysis A 1.0 mg portion of XK-62-2 was suspended in 0.1 ml 6 N HCl, sealed in a small glass tube, and placed in an oven at 110°C for 17~24 hours. The solution was transferred to a round-bottom flask with water and evaporated several times from H_2O to remove excess HCl. The residue was finally suspended in 0.1 ml H_2O and 2 μl was spotted per chromatogram.

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