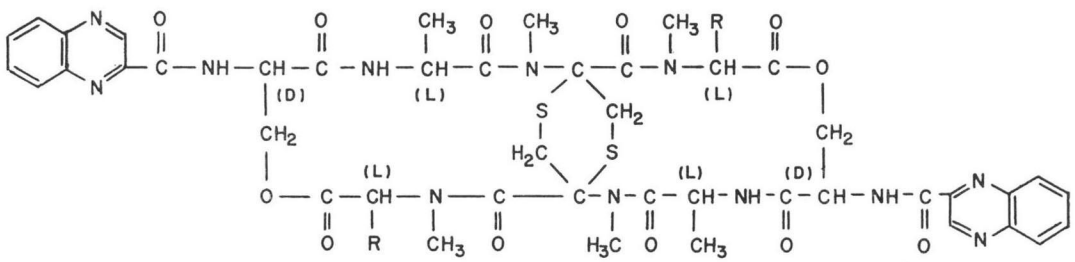
Communications to the editor

STRUCTURE OF QUINOMYCIN ANTIBIOTICS

Sir:

In the course of screening for antitumor antimetabolites,[1] L.J. HANKA of these laboratories submitted lyophilized beer from a control culture lacking antimetabolite activity, for *in vivo* assay against PS-3 leukemia[2] in mice. The lyophilized beer solids significantly prolonged the life span of the leukemic mice and our efforts were enlisted to isolate the active principle. The lipophilic active principle was readily extracted with solvents; mycelial cake was the richest source of active extract. Solvent extracts had reasonable *in vitro* activity and bioautography distinguished the activity from all the antibiotics in the Upjohn collection of known antibiotics. Mycelial extracts were defatted with petroleum ether and purified by silica gel chromatography, preparative thin-layer chromatography, and counter current distribution yielding a homogenous crystalline antibiotic, U-48, 160. Infrared mull spectra suggested a very close relationship with quinomycin A (echinomycin)[3] from which U-48, 160 had already been differentiated by bioautography leaving the probability that the antibiotic was a member of the quinoxaline[4] family. Although the ultraviolet spectrum and optical rotation of U-48, 160 were consistent with the quinoxaline antibiotics,[5,6] NMR spectra, both proton and ^{13}C, apparently differentiated the antibiotic from the reported structures shown (Fig. 1). An apparent S-CH_3 (s, δ 2.1) was inconsistent with published* quinomycin and triostin structures.[5] Since structures of the minor components of the quinoxaline family were based on comparison with quinomycin A, the structure determination[3] of which was accomplished without the benefit of NMR, we investigated the proton and ^{13}C spectra of that antibiotic. To our surprise, the spectra betrayed the presence of the S-CH_3 in a sample which had been found identical to authentic echinomycin** by bioautography on 3 systems as well as comparison of infrared spectra. The 100 MHZ proton spectrum*** (Fig. 2) of quinomycin A shows the presence of 64 protons (4 exchangeable) and agrees well with the expected[3] resonances for quinoxaline, alanine, serine, and N-methylvaline fragments of the molecule. The 2 N-CH_3's adjacent to the bridge section are present but the 2 expected[3] isolated

Fig 1. Reported structures of quinomycins A[3] and C[5].



R = $CH(CH_3)_2$ *Quinomycin A*

R = $CH(CH_3)CH(CH_3)_2$ *Quinomycin C*

* A typographical error was apparent in the structures given in reference 5. The structures given on p. 1540 for the quinomycin antibiotics belong to the triostin family and the structure given on p. 1541 for the triostin antibiotics belongs to the quinomycin family.

** We are grateful to Dr. J. NÜESCH of Ciba-Geigy Ltd., Basel, Switzerland for an authentic sample of echinomycin.

*** The proton NMR spectra in $CDCl_3$ were observed on a Varian XL-100-15 spectrometer operating at 100 MHZ. Tetramethylsilane was used as an internal reference.

Fig. 2. Proton spectra of quinomycin A and U-48, 160 (quinomycin C).

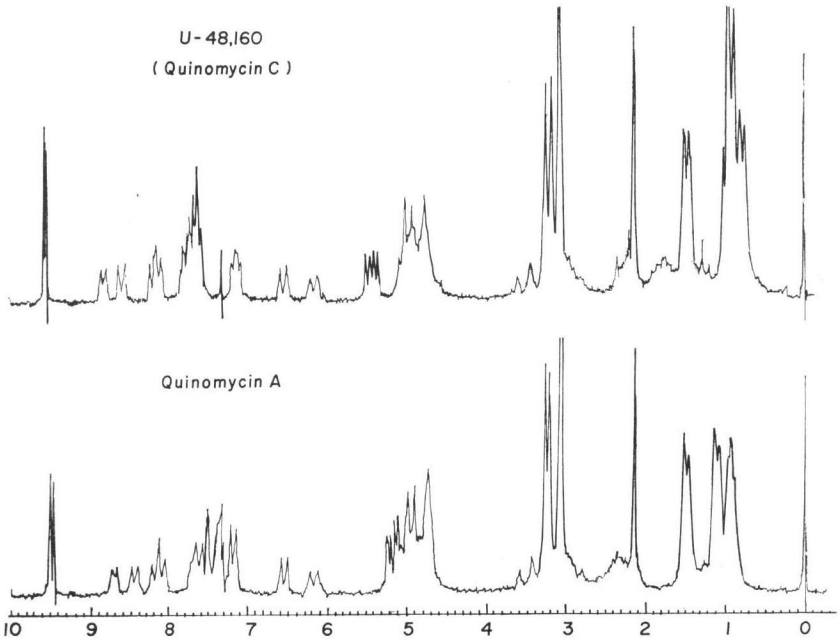
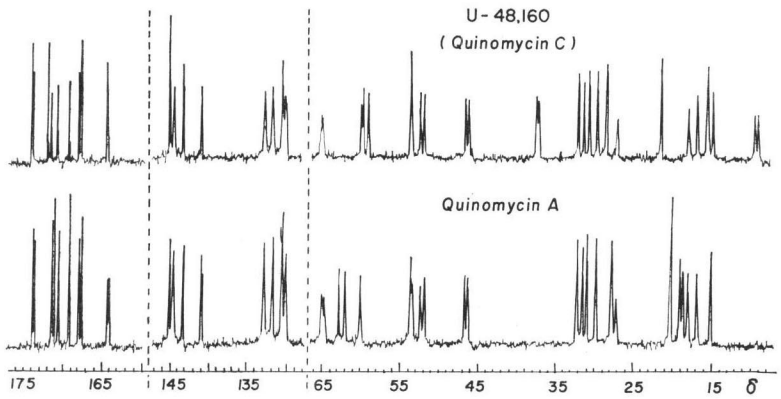


Fig. 3. ^{13}C-Spectra of quinomycin A and U-48, 160 (quinomycin C).



bridge methylenes are absent. Instead a methine (d, $J_{AX}=11.0$, $J_{BX}=0$, δ 6.14) coupled to an S-CH_2 (AB of an ABX, $J_{AB}=-17$, centered at δ 3.16) and a CH (d, J=9.0, δ 6.5) coupled to a CH (under a 9 proton multiplet between δ 4.6 and 5.1) was established by spin decoupling. In addition a signal for an S-CH_3 (s, δ 2.1) is evident.

The ^{13}C spectrum* of quinomycin A (Fig. 3) shows the presence of 51 carbons, including 10 carbonyls, and a lack of C_2 symmetry. The absorptions (Table 1) of the quinoxaline, alanine and serine carbons were assigned by off-resonance decoupling experiments, standard chemical shift data, and comparison to model compounds.[7~10] The absorptions of N-methylvaline were assigned by comparison with the spectrum (Fig. 3) of U-48,160 which we feel is the same as quinomycin C in which the N-methylvaline is replaced by a N, β-

* ^{13}C NMR spectra in $CDCl_3$ were recorded on a Varian XL-100-15 spectrometer operating at 25.16 MHZ in the pulsed FOURIER transform (FT) mode. Tetramethylsilane was used as an internal reference.

Table 1. Chemical shifts[1] and partial assignments of carbon resonances of quinomycin A and U-48, 160 (quinomycin C) in $CDCl_3$.

Alanine	αCH	QA[2]	46.3	46.7	N Methylvaline QA only	αCH		62.0	62.8
		QC[2]	46.3	46.6		βCH		27.8	27.8
	βCH$_3$	QA	17.0	18.1		γCH$_3$		18.8	19.1
		QC	17.1	18.2				20.4	20.4
						NCH$_3$		29.9	32.2
Serine	αCH	QA	51.9	52.3	Nβ Dimethyl leucine[4] QC only	αCH		59.1	59.8
		QC	51.9	52.4		βCH		37.4	37.6
	βCH$_2$	QA	64.6	65.0		γCH		28.6	28.6
		QC	65.0	65.2		γCH$_3$		9.4	9.8
Bridge	CHN	QA	53.4	53.6		δCH$_3$		15.7	15.7
		QC	53.6	53.6				21.7	21.7
	SCH$_2$	QA	27.2	—		NCH$_3$		30.1	32.3
		QC	27.3	—					
	S−CH−S	QA	60.0	—	Quinoxaline	C-2	QA	144.1	144.1
		QC	60.0	—			QC	144.2	144.2
	SCH$_3$	QA	15.3	—		C-3	QA	143.6	143.6
		QC	15.2	—			QC	143.6	143.6
	Adjacent NCH$_3$	QA	31.0	31.6		C-5[5]	QA	132.0	132.0
		QC	31.0	31.6			QC	131.9	131.9
						C-6[5]	QA	130.7	130.7
Carbonyls[3]		QA	164.0	164.1			QC	130.7	130.7
		QC	164.0	164.2		C-7[5]	QA	129.7	129.7
		QA	167.5	167.8			QC	129.6	129.6
		QC	167.3	167.6		C-8[5]	QA	129.2	129.2
		QA	169.1	170.5			QC	129.3	129.3
		QC	168.9	170.3		C-9	QA	139.9	140.1
		QA	171.1	171.3			QC	140.0	140.1
		QC	171.1	171.5		C-10	QA	142.4	142.5
		QA	173.5	173.7			QC	142.4	142.5
		QC	173.4	173.7					

1 Chemical shifts are reported in ppm downfield from internal TMS.

2 QA: quinomycin A; QC: U-48, 160 or quinomycin C.

3 The carbonyl resonances have not yet been satisfactorily assigned.

4 Presumably this leucine derivative is N, γ-dimethylalloisoleucine isolated from quinomycin C hydrolysis[5].

5 The relative assignments have not been made for these carbons.

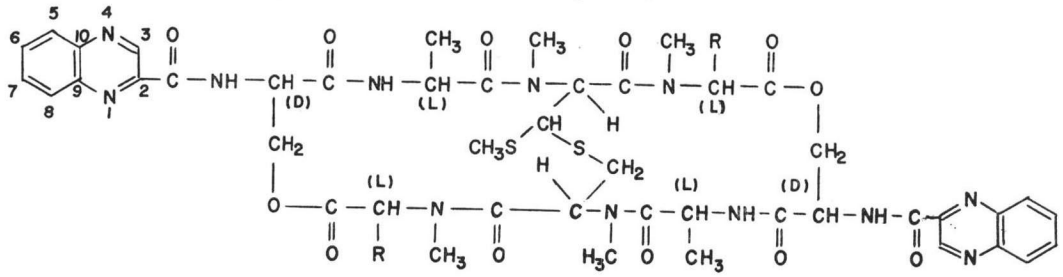
dimethylleucine enantiomer* and by the expected downfield shift of the α CH carbon when a N-CH_3 is present. The bridge N-CH_3's (2Q, δ 31.0, 31.6), S-CH_2 (T, δ 27.2), and S-CH_3 (Q, δ 15.3) were assigned by off-resonance decoupling. The methine carbons at δ 53.6 and 60.0 were assigned to the proton doublets at δ 6.14 and 6.5 respectively by single frequency proton decoupling. The remaining carbon at δ 53.4 was assigned by default. A count of protons from the off-resonance carbon splitting patterns confirms 60 non-exchangeable protons.

Based on these NMR studies and the excellent earlier work of others[3], a revised structure (Fig. 4) is proposed for quinomycin A. Field desorption mass spectrometry afforded further support for this revised structure. A weak M^+ at 1100 was observed ($C_{51}H_{64}N_{12}O_{12}S_2$ requires 1100.421). It is noteworthy that a strong mercaptan odor had been observed during acid hydrolysis of echinomycin[3].

Proton (Fig. 2) and ^{13}C NMR spectra (Fig. 3) of U-48, 160 were very similar to those from quinomycin A; the only apparent difference was the substitution of N, β-dimethylleucine residues for the 2 N-methylvaline residues. This difference is the same as the reported difference[5] between quinomycin C and quinomycin A where 2 N, γ-dimethylalloisoleucine residues in quinomycin C replace the N-methylvaline residues of quinomycin A. Although we were unable to secure an authentic sample of quinomycin C for direct comparison, we feel that U-48, 160 is actually quinomycin C based on (a) the NMR comparison already cited, (b) comparison of the

* Presumably N, γ-dimethylalloisoleucine isolated from quinomycin C hydrolysis (5).

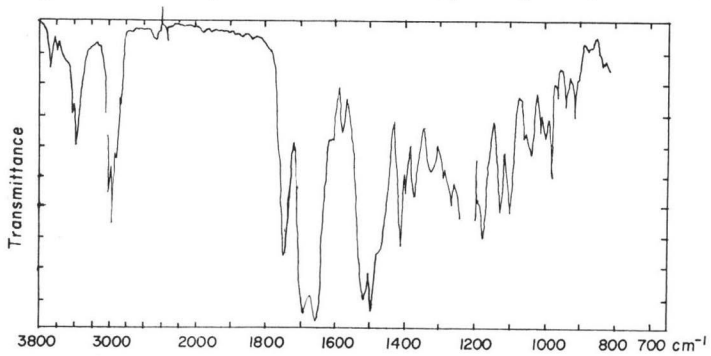
Fig. 4. Revised structures of quinomycins A and C.



R = $CH(CH_3)_2$ *Quinomycin A*

R = $CH(CH_3)CH(CH_3)_2$ *Quinomycin C*

Fig. 5. Infrared spectrum of U-48,160 (quinomycin C) in $CHCl_3$.



infrared solution spectrum* of U-48,160 (Fig. 5) with quinomycin C[5], (c) optical rotation ($[\alpha]_D$ −268; lit. [6] $[\alpha]_D$ −250), and (d) the relative mobilities of U-48,160 and quinomycin A are consistent with the reported[6] relative mobilities of quinomycins A and C.

Accordingly a revised structure (Fig. 4) is proposed for quinomycin C. Field desorption mass spectrometry afforded a weak M^+ at 1156 in support of this structure ($C_{55}H_{72}N_{12}O_{12}S_2$ requires 1156.4833).

Acknowledgements

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* The spectrum was obtained from a 5 % solution in $CHCl_3$ with a Digilab Model FTS-14D Spectrophometer. Gaps in the spectrum are due to areas of total absorption by $CHCl_3$.

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