FOURIER TRANSFORM C-13 NMR ANALYSIS OF SOME FREE AND POTASSIUM-ION COMPLEXED ANTIBIOTICS

M. Ohnishi, M.-C. Fedarko, and John D. Baldeschwieler
Department of Chemistry
Stanford University
Stanford, California 94305

and

L. F. Johnson
NMR Laboratories
Varian Associates
Palo Alto, California 94303

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Fourier transforms of the noise-decoupled, natural abundance ^{13}C nmr free induction decays of the cyclic antibiotic valinomycin and its potassium-ion complex have been obtained at 25.2 MHz. Comparisons are made with ^{13}C nmr spectra taken at 22.6 MHz of the cyclic antibiotic nonactin and the synthetic polyether dicyclohexyl-18-crown-6 and their potassium complexes. The resonances of the carbonyls directly coordinating the potassium ion in valinomycin and nonactin shift downfield about 4 ppm upon complex formation. Smaller but comparable shifts in both up- and downfield directions for carbons away from the binding site are observed by comparisons of the spectra of free and complexed nonactin and the polyether. This suggests that conformational rearrangements of the molecule as a whole can compete with direct interactions between carbons and the potassium ion in determining ^{13}C chemical shift differences between the free and complexed species.

Various macrocyclic antibiotics are known to induce ion permeation in mitochondria and to transport ions selectively by acting as neutral ion carriers.[1] These molecules have ring oxygens (carbonyls or ethers) arranged so that they can replace the hydration shell of the cation. We have obtained the ^{13}C nmr spectra of several potassium-ion complexing antibiotics (valinomycin,[2-5] nonactin,[6-8] and the synthetic polyether dicyclohexyl-18-crown-6[9-11]) and have observed that the conformational changes caused by formation of the ion complexes are re-

flected in changes in the ^{13}C chemical shifts. Chemical shift differences between corresponding carbons in the free and complexed antibiotics amount to as much as several ppm.

Noise-decoupled, natural abundance, pulsed ^{13}C nmr spectra were obtained at 25.2 MHz using a Varian XL-100 spectrometer. The ^{13}C chemical shifts were measured in ppm downfield from internal tetramethylsilane and are denoted by δ_c. Natural abundance CW ^{13}C nmr spectra were also obtained using a Varian XL-100 spectrometer interfaced with a C-1024 time-averaging computer. Valinomycin, $C_{54}H_{90}N_6O_{18}$, was obtained from Calbiochem; nonactin, $C_{40}H_{64}O_{12}$, from Dr. B. Stearns of the Squibb Institute for Medical Research; and the polyether, $C_{20}H_{36}O_6$, from Dr. H. K. Frensdorff of E. I. du Pont de Nemours.

The chemical shifts of an 0.07 M (200 mg/2.5 ml) d_4-methanol solution of valinomycin are shown in Table I. The division of the spectrum into a methyl-carbon region (δ_c~20), a β-carbon region (δ_c~30), an α-carbon region (δ_c 60-80), and a carbonyl carbon region (δ_c~170) is straightforward.

There are three lines in the valinomycin α-carbon region. Because these lines are so well separated they can be assigned by comparison with the ^{13}C nmr spectra of the component acids (Table I), even though there are chemical shift deviations of 2 to 6 ppm between the acids and the corresponding valinomycin lines. The highest field line (δ_c 59.8) is more intense than the others and is assigned to the D- and L-valine residues. The lines at δ_c 80.0 and 71.7 are assigned to the α-carbons of the D-hydroxyisovaleric and L-lactic acid residues, respectively. Straight-chain model compounds were synthesized to approximate the environment of each residue in valinomycin (lactate analogue = DL-N-methyl-2-acetoxypropionamide, hydroxyisovalerate analogue = DL-N-methyl α-acetoxyisovaleramide, valine analogue = N-acetyl DL-valine methyl ester). The ^{13}C nmr spectra of these analogues were to help in identifying the antibiotic resonances. As can be seen in Table I, the α-carbon chemical shift of each compound agreed with that in valinomycin to within 0.6 ppm.

Table I

^{13}C Chemical Shifts (ppm from TMS) of Valinomycin, Valinomycin-K^+, and Model Compounds

	DL Lactic Acid[a]	DL Hydroxy-isovaleric Acid[a]	DL Valine[b]	Lactate Analogue	Hydroxy-isovalerate Analogue[a]	Valine Analogue	Valino-mycin	Valino-mycin-K^+	$\Delta\delta_C$
	178.2	177.4	175.8	173.6 (amide)	172.6 (amide)	173.6 (acyl)	173.1	177.0	3.9
C=O				171.3 (acyl)	171.9 (acyl)	173.1 (amide)	172.5	174.1	1.6
							171.9	176.5	4.6
							171.5	172.6	1.1
		76.3			79.5		80.0	80.9	0.9
α-CH	67.6			71.3			71.7	72.5	0.8
								63.0	3.2
			61.6			59.2	59.8	62.8	3.0
		33.1			31.7		31.7	31.5	-0.2
β-CH			30.2			31.5	31.1	29.7	-1.4
							30.8	29.6	-1.2
							19.7	20.6	
	20.6	19.1	19.1	26.3 ($CONHCH_3$)	26.1 ($CONHCH_3$)	52.4 ($COOCH_3$)	19.6	20.5	
		16.8	17.9	20.7 ($OCOCH_3$)	20.6 ($OCOCH_3$)	22.3 ($NHCOCH_3$)	19.3	19.9	
CH_3				18.1	19.0	19.3	19.2	19.3	
					17.5	18.5	18.8	19.3	
							17.7	17.8	
							17.5	17.2	

[a] J. Schaefer, unpublished results.

[b] W. Horsley, et al, JACS, 92, 680 (1970).

The β-CH region was also assigned. In the component acids and the straight-chain analogues, the β-CH of hydroxyisovalerate was downfield of the corresponding valine resonance. This, combined with the fact that in valinomycin the two upfield β-CH resonances were close and almost coalesced on forming the K^+ - complex, strongly suggested the assignment of δ_c 31.7 belonging

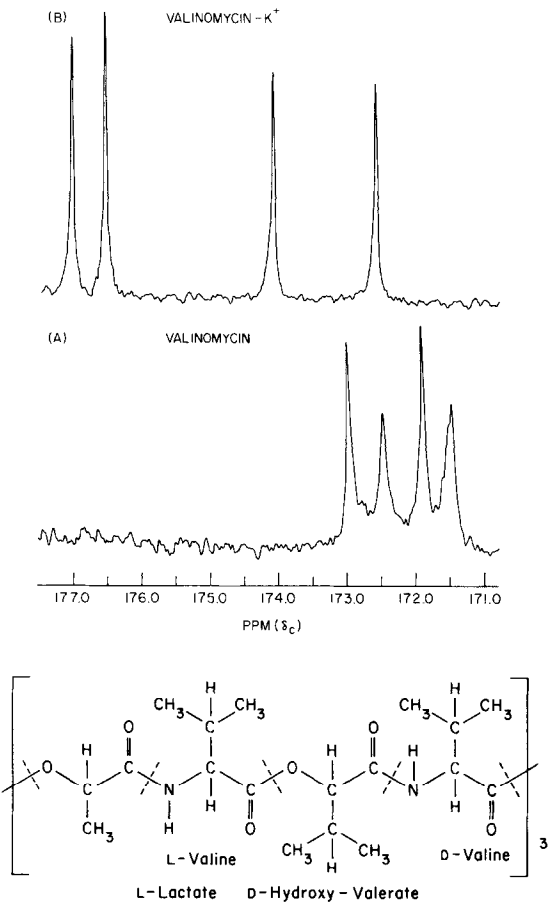


Figure 1. Proton-decoupled natural-abundance ^{13}C fourier transform nmr spectra of

(a) valinomycin carbonyl region, 250 Hz sweep width, 11,094 transients, 1.0 sec acquisition time;

(b) valinomycin-K^+ carbonyl region, 250 Hz sweep width, 33,190 transients, 1.0 sec acquisition time.

Chemical shifts are given with respect to an internal standard of TMS.

to the hydroxyisovalerate carbon, and δ_c 30.8, 31.1 belonging to the valine carbons. No attempt was made to assign the seven lines in the methyl region (δ_c 19.7-17.5).

Assignments of the four carbonyl carbons in valinomycin were based on the following empirical peak height argument. There are two types of carbonyls in this compound: N-linked carbonyls (lactate, hydroxyisovalerate), and O-linked carbonyls (both valines). ^{14}N coupling would be expected to broaden neighboring carbonyls; hence, N-linked carbons would have a smaller peak height than the O-linked carbons. There could also be some contribution to the reduced peak height by coupling of the carbonyl carbon with the amide deuterium which would not be removed by proton noise decoupling.[12] As seen in Fig. 1a, there are clearly two types of carbonyls. The peaks at 173.1 and 171.9 are slightly narrower and higher, and hence are assigned to the valine residues. In an attempt to strengthen the assignment, the carbonyl shifts of the straight-chain analogues were examined. Each analogue has N-linked and O-linked carbonyls which can be distinguished in the ^{13}C nmr spectrum by peak height. The range of corresponding chemical shifts was 172.6 to 173.6 ppm, with hydroxyisovalerate the upfield line and lactate and the valines around the same value. These shifts are all downfield of those observed in valinomycin (171.5 to 173.1 ppm). At most the analogues suggest the ordering between the lactate and hydroxyisovalerate resonances (i.e., in valinomycin the farthest upfield carbonyl peak belongs to hydroxyisovalerate, and the middle small peak to the lactate).

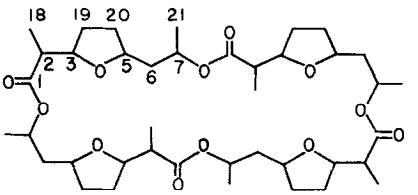
The chemical shifts of an 0.1 M methylene chloride solution of nonactin are seen in Table II. Using simple additivity considerations and the multiplet structure of the undecoupled spectrum, one can divide the nonactin lines into a carbonyl carbon region (δ_c~175), an oxygen-bonded -CH-O- carbon region (δ_c 69-81), C-2 (δ_c 45.7), C-6 (δ_c 42.7), a ring methylene carbon region (δ_c~30), and a methyl carbon region (δ_c 13-21).

The chemical shifts of an 0.3 M methylene chloride solution

Table II

^{13}C Chemical Shifts (in ppm from TMS) of nonactin and its K^+ - complex as 0.3 M solutions in CD_2Cl_2[a]

NONACTIN	NONACTIN-K^+	$\Delta\delta_c$	CARBON ASSIGNMENT
174.2	177.9	3.7	1
80.7	82.4	1.7	7
76.8	75.0	-1.8	3,5
69.4	67.5	-1.9	
45.7	46.6	0.9	2
42.7	44.9	2.2	6
31.9	31.9	0	19,20
28.5	29.3	0.8	
20.7	21.1	0.4	18,21
12.9	15.5	2.6	



[a] J. Schaefer, unpublished results

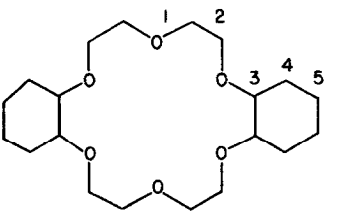
of dicyclohexyl-18-crown-6 are given in Table III. The five major lines of the polyether spectrum can be divided into two classes with the three lines at lower field assigned to carbons directly bonded to oxygens (the lowest belonging to methyne C-3), and the higher field lines assigned to the ring methylenes. Smaller lines accompany each of the major peaks in the spectrum. These may arise from the different possible isomers with respect to substitution on the cyclohexane rings.

There are substantial differences between the ^{13}C nmr spectra

Table III

^{13}C Chemical Shifts (ppm from TMS) of the Polyether Dicyclohexyl-18-crown-6 and its K^+ Complex 0.3 M in CD_2Cl_2

POLYETHER	POLYETHER-K^+	$\Delta\delta_c$	CARBON ASSIGNMENT
77.6	78.1	0.5	3
71.2	70.7	-0.5	1,2
68.2	67.3	-0.9	
28.0	26.3	-1.7	4,5
22.4	22.0	-0.4	



[a]J. Schaefer, unpublished results

of the free and potassium-ion complexed compounds, as illustrated in Tables I-III. The carbonyl carbons attached to oxygens directly coordinating the potassium ion are shifted downfield considerably as might be predicted from carbonyl pi-bond polarity arguments.[13,14] The nonactin carbonyl resonance is shifted 3.7 ppm downfield. Using the peak heights to assign carbonyls in the complexed valinomycin spectrum (Fig. 1b), one finds that the two valine carbonyls are shifted 3.9 and 4.6 ppm downfield, while the other two residues undergo lesser downfield shifts (1.1 and 1.6 ppm). X-ray[15] and ^1H nmr[2-5] studies have shown that the valine carbonyls coordinate the potassium ion in an octahedral arrangement, while the carbonyls of the other two residues are involved in intramolecular hydrogen bonding and not directly

attached to the ion. The ^{13}C nmr data is consistent with such a structure: only two large valinomycin carbonyl carbon shifts are observed on complex formation; the α-carbon resonances of valine are shifted 3 ppm downfield while those of the other residues are affected much less; similarly, the valine β-carbon resonances are shifted 1.2 and 1.4 ppm upfield while the hydroxy-isovalerate peak barely moves.

However, this kind of a simple correlation of structure with the influence of complex formation on the ^{13}C nmr chemical shifts may be somewhat misleading. In the crown polyether the carbons attached to ether oxygens which coordinate the potassium ion change by less than 1 ppm upon complex formation. The corresponding ether-linked carbons in nonactin (jointly coordinating the ion with the carbonyls) shift by much more (1.8, -1.8, -1.9 ppm). Furthermore, in the spectra of both nonactin and the polyether, the resonance of a methylene or methyl carbon removed from the site of the potassium ion is shifted by a large amount upon complex formation: 2.6 ppm for a methyl carbon in nonactin, and -1.7 ppm for one of the cyclohexyl methylene carbons in the polyether. These results suggest that at least for some carbons, induced conformational rearrangements of the molecule as a whole are as important as more direct interactions with the potassium ion in determining potentially informative relative ^{13}C nmr chemical shifts.

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