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CARBON-13 N M R SPECTRA OF THE ANTIBIOTIC BUTIROSIN A, AND RELATED AMINOGLYCOSIDES

PETER W K. WOO AND ROGER D WESTLAND

Chemistry Department, Research and Development Division, Parke, Davis and Company, Ann Arbor, Michigan 48106 (U. S A)

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ABSTRACT

From the c m r spectra of the individual components of the aminoglycoside antibiotic butirosin A and other pertinent, simpler compounds, the spectra of progressively more complex molecules consisting of different combinations of these components were analyzed The effects of protonation and *N*-trifluoroacetylation were studied The potential usefulness of the c m r technique for structural elucidation and characterization of aminoglycoside antibiotics is indicated

INTRODUCTION

In recent years, Fourier-transformed carbon-13 nuclear magnetic resonance (c m r) spectroscopy has been applied to various classes of compounds, such as steroids[1], nucleosides[2], amino acids and peptides[3], inositols[4], and mono-[5] and oligo-saccharides[6]. However, the application of this technique to aminoglycoside antibiotics, a therapeutically important class of compounds, has not been reported

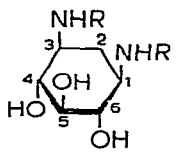
In connection with a program on the structural modification of aminoglycoside antibiotics, it became evident that c m r. spectroscopy might serve as an important and necessary complement to the other physical methods for the characterization and structural elucidation of this class of compounds A typical example of an aminoglycoside antibiotic is butirosin A[7–10] (8a), $C_{21}H_{41}N_5O_{12}$, the subject of the present study. Butirosins lack definite melting points, characteristic u v. absorption, and definitive i r spectra Although both p m.r and mass spectrometry provided crucial information during structural elucidation of butirosins[8,9], the size of the molecule gave rise to the problem of overlapping peaks, especially in the p m r. spectrum On the other hand, c m r , besides providing a different type of structural information, is expected to show a high degree of resolution because carbon resonances occur over a range exceeding 200 p p m [11].

RESULTS AND DISCUSSION

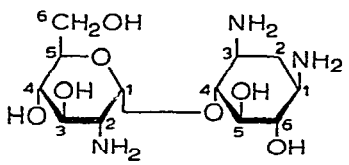
An initial assumption was that the c m.r. spectrum of a complex aminoglycoside should be essentially a composite of the spectra of its simpler components, with adjustment for the effect of bonding between the various components. This approach

led to the successful c m r.-spectral correlation of a number of molecules studied in this work. From the c m r spectra of the individual structural components of butirosin, as well as of other pertinent, simpler compounds, the spectra of progressively more-complex molecules, consisting of different combinations of these components, were analyzed. Finally, the peaks in the spectrum of butirosin A were assigned (although tentatively for some) with individual assignments for twelve carbon atoms and one group assignment each for two, three, and four carbon atoms. In addition, the effects of protonation and *N*-trifluoroacetylation were briefly studied These results should provide an initial framework for further application of the c m r technique to other aminoglycoside antibiotics

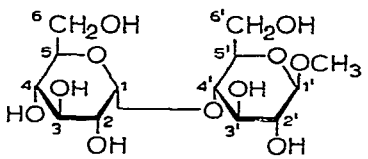
The c m r spectrum of deoxystreptamine[7] (D) (**1a**) in deuterium oxide (Table I) shows four peaks, at 113 9, 115 9, 141.1, and 155.6 p p m upfield from external carbon disulfide, with relative peak-height ratios of 2 5 1 2 0 4, respectively[11]. These chemical-shift and peak-height values allow straightforward peak assignments



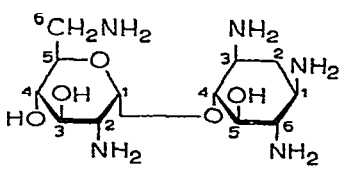
1a Deoxystreptamine, D, R=H
b D 2HCl, R=H·HCl
c D $^2H_2CO_3$, R=H·1/2 $^2H_2CO_3$



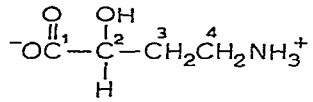
2 Paromamine, GlcN–D



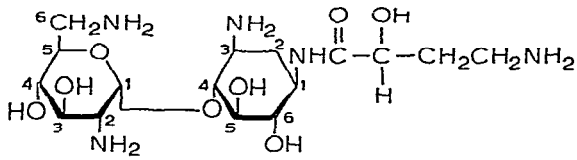
3 Methyl β-maltoside, Glc–Glc'–OCH_3



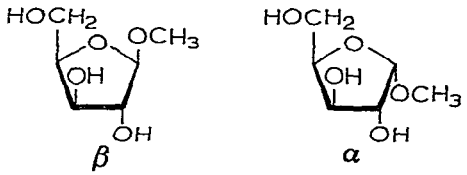
4 Neamine, Nc–D



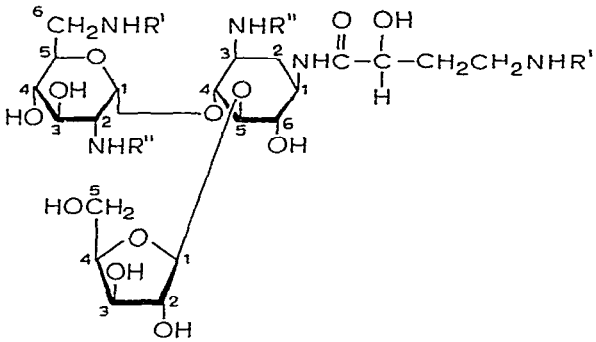
5 Ab



6 Butirosin-P, Nc-D-Ab



7 Xylf-OCH_3



8a Butirosin A, Nc-D(Xylf)-Ab, R'=R"=H

b (TFA)$_4$butirosin A, R'=R"= $-\overset{O}{\overset{\|}{C}}CF_3$

c Butirosin A $2H_2SO_4$, R'=R"=H $1/2\,H_2SO_4$

d (TFA)$_2$butirosin A H_2SO_4, R'=H $1\,2H_2SO_4$, R"= $-\overset{O}{\overset{\|}{C}}CF_3$

for **1a** from two considerations First of all, **1a** possesses a plane of symmetry through carbon atoms D2* and D5, with the result that carbon atom D1 is equivalent to D3, and D4 equivalent to D6 Secondly, previous studies have shown that substitution of a carbon atom by hydroxy, amino, or alkyl substituents all p.oduce downfield

*The symbolism used denotes the position number of the carbon atoms on the indicated fragment, as shown in the formula charts

TABLE I

^{13}C CHEMICAL-SHIFT ASSIGNMENTS FOR BUTIROSIN A AND RELATED COMPOUNDS[a–d]

Carbon atom[e]		**1a**, *D* 5, *Ab* 7, *Xylf-OMe*	**1b**, *D 2HCl*	**1c**, *D*·$^2H_2CO_3$	**2**, *GlcN-D*	**4**, *Nc-D*	**6**, *Nc-D-Ab*	**8a**, *Nc-D-Ab* | *Xylf*	**8b**, $(TFA)_4$-*butirosin A*	**8c**, *Butirosin A* $2H_2SO_4$	**8d**, $(TFA)_2$-*butirosin A* H_2SO_4
		1a, D									
	1	141.1	141 1	141 3	[141 2 142 2]	[141 2 142 4]	[141 9 142 3]	[141 4 142 1]		[142 3 142 7]	142 4 142,4
D	3	141 1	141 1	141 3							
	2	155 6	163 2	161 4	155 7	156 4	[157 6, 158 1]	[157 0, 157 5]	[158 5, 159 2]	160 6	[160 2, 160 8]
	4	113 9	119 1	117 5	103 8	105 4	104 9	106 9	106 8	[108 8, 110 8]	[109.5, 111 0]
	6	113 9	119.1	117 5	114 1	114 6	[111 4, 117 9 118 6] t	[117 0, 117 2 118 4] t	[117 1, 118 6 120.3, 120 8]	[117 1, 117.7]	[117 2, 118 0]
	5	115 9	116.7	116 0	115 7	115 7	115 5	[109 6, 109 8 110 7, 111 0]	[110 7, 111 4 112 2]	[108,8, 110 8]	[109 5, 111.0]
	1				90 6	91 3	90 8	92.7	94 3	96 8	96 2
Glc,	2				136 2	136.5	136 3	135,9	137 0	137 7	137 9
GlcN	3				[117 8 118 7] t	[118 3 119 1] t	[117 4 117 9, 118.6] t	[117 0 117 2, 118 4] t	[117 1 118 6, 120 3 120 8]	[122 4 123 2] t	[121 7 123 4] t
or	5										
Nc	4				121 7 t	120 2 t	120 1 t	120 1 t		120 4 t	121 1 t
	6				130 8	150 4	149 8	150 0	151 4	151 0	151 8

		5, Ab					
	1	11 53	15 42	15 6	17 03	16 06	16 28
Ab	2	121 3	121 7	121 7	122 6	121 9	122 0
	3	160 3	156 6	157 5	[158 5, 159 2]	160 6	[160 2, 160 8]
	4	154 6	154 7	154 8	156 2	154 9	155 0
		7, Xyl*f* OMe					
		β *α*					
	1	83 0, 89 7		81 5	80 1 t	79 8	80 0
	2	[109 2, 111.8]		[109 6	110 7	[105 5	[105 6
Xyl*f*	3	113 4		109 8, 110 7	111 4	115 7, 117 1	115 7, 117.2
	4	114 9, 116 6]		111 0]	112.2	117 7]	118 0]
	5	130 4, 131 1		131 0	130 7	131 0	131 4
	OMe	136 4, 135 9					
	O 1				[33 7, 34 9]		33 7
	‖				35 4]		
CF_3C-	2				[~57 5, 69.8]		
					82 5t, —]		

[a]In parts per million upfield from external CS_2 [b]Values in a bracket represent a group assignment for several carbon atoms or alternative values for one carbon atom [c]Tentative assignments are designated by "t" [d]All compounds were determined in deuterium oxide except **8b**, which was determined in methanol-d_4 [e]Individual carbon atoms (1, 2, 3, etc) in the various components (D, Glc, GlcN, etc.).

shifts, in decreasing order of magnitude (for example, the shift parameters for an α carbon atom having –OH, $-NH_2$, and $-CH_3$ as substituent groups are reported[3b] to be −48 5, −29, and −9 1 ±0 1 p p m, respectively) Accordingly, in the spectrum of **1a**, of the two peaks at the lowest field, the more intense one (relative peak-height, ≥ 5) at 113 9 p p m can be assigned to carbon atoms D4 and D6, and the less-intense one (relative height, 1) at 115 9 to carbon atom D5, each of these atoms being one that carries one hydroxyl and two alkyl substituents The strong peak (relative height, 2) at intermediate field (141 1 p p m) can be assigned to D1 and D3, each of these carbon atoms being one that carries one amino and two alkyl substituents The remaining weak peak (relative height, 0 4) at highest field (155 6 p p m) can be assigned to D2, a carbon atom carrying two alkyl substituents

The spectrum of deoxystreptamine dihydrochloride (D 2HCl) (**1b**) shows the influence of protonation of the amino groups The peak for D4 and D6, each β to an amino group, now appears at 119 1 p p m (shift of +5 2 p p m from the base **1a**) The peak for D5, γ to two amino groups, appears at 116 7 p p m (shift of +0 8) The peak for D1 and D3, each α to one amino and γ to one amino group, is observed at 141 1 p p m (no shift) The peak for D2, β to two amino groups, appears at 163 2 p p m (shift of +7 6) Thus, the influence of protonation is greatest on the β carbon atom (Cβ), and, except for the lack of shift on the α carbon atom (Cα), is in general agreement with the results reported[3a,b] for the protonation of amines (upfield shift of 2 5 to 3 5 p p m for Cα, 3 9 p p m for Cβ) The influence is additive to an extent, although not entirely, as the shift for D2 (β to two amino groups) is about 1 5 times the shift for D1 or D3 (β to one amino group)

It is also necessary to study the effect of protonation by a weak acid, because inadvertent formation of the carbonate sometimes occurs during the preparation of the free-base form of an aminoglycoside antibiotic The c m r spectrum of deoxystreptamine carbonate (**1c**) in deuterium oxide saturated with carbon dioxide shows peaks at 116 0 p p m for carbon atom D5 (shift of +0 1 p p m from the free base **1a**), 117 5 for D4 and D6 (shift of +3 6), 141 3 for D1 and D3 (shift of +0 2), and 161 4 p p m for D2 (shift of +5 8) Thus, the shifts caused by formation of the carbonate are appreciable for the carbon atoms β to the amino groups, although the magnitude of the shifts are less than that caused by protonation with hydrogen chloride, a strong acid

Paromamine[1,2] (GlcN-D) (**2**) consists of 2-amino-2-deoxy-D-glucose (GlcN) glycosidically linked to the hydroxyl group at carbon atom D4 of deoxystreptamine (D) (**1a**) The effect on the c m r absorptions of the D carbon atoms should parallel those established for the methylation of hydroxyl groups in inositols (namely, the effect on an α carbon atom, a shift of −7 to −10 p p m ; on a β carbon atom bearing an axial hydroxyl group, +4 5 p p m for each *O*-methyl group, on a β carbon atom having an equatorial hydroxyl group, *ca* −0 5 p p m ; and on γ and δ carbon atoms, less than ±0 3 p p m)[4] Thus, as compared with **1a**, carbon atom D4 of **2** should show a downfield shift and is assigned to the peak at 103 8 p p m, a shift of −10 p p m The other D carbons of **2** should show minimal shifts, and thus

the peak at 114 1 is assigned to D6, 115 7 to D5, 141 2 and 142 2 to D3 and D1 as a group, and 155 7 p p m (weak) to D2

The remaining peaks in the spectrum of **2** are assigned to the GlcN moiety of **2** It may be noted that the GlcN moiety is quite analogous to a corresponding moiety Glc in methyl β-maltoside[6a] (Glc-Glc′-OMe) (**3**), except for the presence of an amino group at GlcN2 instead of a hydroxyl group at Glc2 Among the carbon atoms of the GlcN moiety of **2**, GlcN1 is expected to resonate at the lowest field, by analogy with the methylation shifts already mentioned, and is thus assigned to the peak at 90 6 p p m Carbon atom GlcN2, carrying an amino group, is expected to resonate at the highest field, and is thus assigned to the peak at 136 2 p p m Carbon atom GlcN6 should resonate at a higher field than the more highly substituted GlcN3, GlcN5, and GlcN4 atoms, and is thus assigned to the peak at 130 8 p p m (*cf* [6a] Glc6 of **3** at 131 8[6a]) The two peaks at 117 8 and 118 7 p p m are tentatively assigned collectively to GlcN3 and GlcN5, and that at 121 7 is tentatively assigned to GlcN4, based on the tentative literature assignment[6a] for Glc3 and Glc5 (119 9, 120 8 p p m , group assignment) and Glc4 (123 0 p p m) in **3**

Neamine[7] (Nc-D, neomycin A) (**4**) consists of 2,6-diamino-2,6-dideoxy-D-glucose (neosamine C, Nc) linked glycosidically to D4 of deoxystreptamine (**1a**) (D) It differs from paromamine (**2**) (GlcN-D) in having an amino group at Nc6 instead of the hydroxyl group at GlcN6 of **2** The peak for Nc6 of **4** is observed at 150 4 p p m , as compared with 130 8 p p m for GlcN6 of **2** The peak positions for the rest of the carbon atoms in **4** are almost the same as those of the corresponding carbon atoms in **2**, and are therefore readily assigned accordingly

Butirosin possesses a structural component unique among aminoglycoside antibiotics obtained by fermentation, namely (*S*)-(−)-4-amino-2-hydroxybutyric acid[7] (Ab) (**5**) The spectrum of this acid in deuterium oxide, undoubtedly in the zwitterionic form, shows four peaks, as follows Ab1, 11 53, Ab2, 121 3; Ab4, 154 6, and Ab3, 160 3 p p m

Butirosin-P[13] (Nc-D-Ab) (**6**) consists of the acid **5** (Ab) attached to the amino group at D1 of neamine (**4**) (Nc-D) through an amide linkage Peak assignments for butirosin-P were accomplished by comparison with the spectra of **4** and **5** The peak at 121 7 p p m is assigned to Ab2 in butirosin-P on the basis of peak position (121 3 p p m in free acid **5**) and intensity, characteristically much higher than the other peaks at 115 5 to 120 1 p p m , assigned as a group to D6, D5, Nc3, Nc5, and Nc4 The peak at 154 7 p p m is similarly assigned to Ab4 on the basis of peak position and intensity A peak at 156 6 p p m is assigned to Ab3, which would represent a downfield shift of 3 9 p p m from the free, zwitterionic acid **5**, an extent reasonable for a carbon atom β to an amino group[3b] The assignment of a peak at 157 6 or 158 1 p p m (weak peaks) to D2 is tentative, because of the presence of extraneous, weak signals

Butirosin A (Nc-D-Ab) (**8a**) differs from butirosin-P (**6**) (Nc-D-Ab) in having a

Xyl*f*

pentose, D-xylose (Xyl*f*), attached as a *β*-furanosyl residue[9] to the D5 hydroxyl group of butirosin-P Comparison of the spectrum of **8a** with that of **6** reveals a large number of peaks common to both spectra, assignments for these peaks are therefore readily made One new peak at 81 5 p p m is assigned to Xyl*f*1 Four new peaks, at 109 6 to 111 0 p p m are assigned as a group to Xyl*f*2, Xyl*f*3, Xyl*f*4, and D5, indicating that D5 shows a downfield shift through glycosylation of 4 5 to 5 9 p p m (from 115 5 p p m in butirosin-P) A new peak at 131.0 p p m is assigned to Xyl*f*5

For confirmation of these assignments to the Xyl*f* carbon atoms in butirosin (**8a**), the spectrum of a mixture of methyl *α*(and *β*)-xylofuranoside (Xyl*f*-OCH_3) (**7**) was used for comparison The Xyl*f*1 peak at 83 0 p p m of the anomeric mixture **7**, being quite close to the *β*-anomeric[9] Xyl*f*1 peak at 81 5 p p m in **8a**, is assigned to the *β* anomer, whereas the Xyl*f*1 peak further downfield (at 89 9 p p m) was assigned to the *α* anomer As indicated by the relative peak heights of Xyl*f*1 (1 4 1), the *β* anomer preponderates in the mixture This peak-height difference was further utilized to differentiate the Xyl*f*5 and OCH_3 peaks of the two anomers Being more highly substituted by alkyl groups, the Xyl*f*5 peaks should appear at lower field than the OCH_3 peaks Accordingly, the double peaks at 130 4 and 131 1 p p m were assigned to Xyl*f*5 (*β* and *α* anomers, respectively), whereas the double peaks at 135 9 and 136 4 p p m were assigned to the OCH_3 carbon atoms of the *α* and *β* anomers, respectively The value of 130 4 p p m for Xyl*f*5 (*β* anomer) agrees well with 131 0 p p m , assigned to Xyl*f*5 of **8a**, as indicated in the previous paragraph

The c m r spectra of several derivatives of butirosin A were studied to observe the effect of *N*-acylation[14] and protonation

The spectrum of *N*,*N*′,*N*,″*N*‴-tetrakis(trifluoroacetyl)butirosin A[15] (**8b**) was obtained in deuterated methanol (CD_3OD) Thus, comparison with the spectrum of butirosin A (**8a**) recorded in deuterium oxide may not be entirely valid Nevertheless, the observed changes are minimal, and in no instance exceed 2 p p m Carbon atoms Nc2, Nc6 and Ab4 show upfield shifts of 1 1, 1 4, and 1 4 p p m , respectively Signals of carbon atoms D1 and D3 could not be observed as they were masked by the solvent peaks

In the spectrum of butirosin A disulfate (**8c**), the carbon atoms *β* to the primary amino group (C*β*) are expected to show the largest upfield shifts as compared with butirosin A base (**8a**), whereas carbon atoms *α* to the primary amino groups (C*α*) should show smaller upfield shifts[3a,b] [compare protonation of deoxystreptamine (**1**) already discussed]. Based on these premises, the shifts (with reference to **8a**) assigned to C*β* are, approximately 5 p p m for Nc3 and Nc5, 4 p p m for Nc1 and D4, 3 p p m for Ab3, and 2 p p m for D2 The shifts assigned to C*α* are 1 8 p p m for Nc2; *ca* 1 p p m for Nc6 and D3, 0 p p m for Ab4 If these assignments are correct, then one of the Xyl*f*2, Xyl*f*3, and Xyl*f*4 carbon atoms must display a downfield shift of about 4 to 5 p p m , whereas the other two would show upfield shifts of 4 7 to 8 p p m

The spectrum of N^2,N^3-bis(trifluoroacetyl)butirosin A sulfate[15] (**8d**) is essentially

the same as that of butirosin A disulfate (**8c**) Apparently, most of the changes appear to be not more than 1 0 p p m

We are not certain about the causes of the large shifts for Xyl*f*1, Xyl*f*3, and Xyl*f*4 (with reference to **8a**) observed in **8c** and **8d** They appear to be associated with the protonation of the amino groups at Nc6 or Ab4, rather than those at Nc2 and D3 They may have originated from changes in hydrogen bonding (and hence the electron density) of the C–O bonds of Xyl*f*2, Xyl*f*3, and Xyl*f*4, or from changes in the spatial relationship of these carbon atoms with the rest of the molecule, or from changes in the conformation of the xylofuranose ring It may be noted, too, that some neutral carbohydrate compounds have been reported[6a] to show c m r shifts upon changing the pH of the solution from neutral to alkaline

In conclusion, the excellent resolution in the c m r spectrum of butirosin A (**8a**) (20 peaks for 21 carbon atoms), and the relative ease with which peak assignments or correlations with related compounds can be made, indicate the potential usefulness of c m r spectroscopy for structural elucidation and characterization of aminoglycoside antibiotics Results from the present study show that the technique should be especially useful for ascertaining changes in the chemical environment (for example, variations in hetero atoms at the α position) close to a particular carbon atom under investigation, they also indicate the feasibility of biosynthetic studies of these compounds using carbon-13 labeling[16]

EXPERIMENTAL

Spectra were recorded with a Bruker HFX-10 instrument at 22 6 MHz for ^{13}C, by using a 90-MHz decoupling frequency for broad-band ^{13}C-H decoupling Samples were examined in deuterium oxide in a 10-mm (o d) tube (except for **8b** which was determined in methanol-d_4) A mixture of hexafluorobenzene and carbon disulfide in a smaller, concentric tube provided the fluorine lock-signal and the external standard for the carbon-13 signals Chemical shifts are reported in p p m upfield from carbon disulfide

In a typical measurement, a solution of 564 mg (*ca* 1 mmol) of butirosin A in 1 8 ml of deuterium oxide was used The instrument conditions were 12,581 scans, pulse-width 80 μsec , dwell time 100 μsec , delay time 1/16 μsec There were 4095 signal points at a sweep width of 5000 Hz All samples were examined at room temperature

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