FORTIMICINS A AND B, NEW AMINOGLYCOSIDE ANTIBIOTICS

III. STRUCTURAL IDENTIFICATION*

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The structures of fortimicins A and B have been determined by PMR, CMR, mass spectra and CD combined with chemical degradations. Both antibiotics are pseudodisaccharides and incorporate a novel aminocyclitol, fortamine. Incontrast to the diaminocyclitol moieties of known aminoglycosides, fortamine is a 1,4-diamine, contains both *N*- and *O*-methyl groups and possesses *chiro* stereochemistry. Both antibiotics are glycosides of 6-*epi*-purpurosamine B, but fortimicin A differs from fortimicin B by being a glycyl amide.

Fortimicins A and B have been shown by their general physical properties and method of isolation to be two members of a novel family of aminoglycoside antibiotics.[1,2] Their specific chemical structures, which are unusual to this class in several details, have been determined by the integrated application of physical methods and chemical manipulation. The discussion that follows will outline, in detail, evidence which firmly establishes their structures.

Fortimicin B

The isolation and purification of sufficient quantities of fortimicin B free base for spectroscopic investigation was central to the structure proof of these antibiotics. The 100 MHz PMR spectrum of fortimicin B free base in D_2O solution is shown in Fig. 1 and indicates that this compound has a relatively simple structure. The PMR parameters are summarized in Table 1. Only a single anomeric resonance is observed at 5.50 ppm suggesting that fortimicin B is a pseudodisaccharide. The observed small coupling is consistent with the α-D anomeric configuration usually associated with this class of antibiotics. Prominent singlets at 3.92 and 2.85 ppm indicate the presence of *O*- and *N*-methyl groups.

A methyl doublet is observed at highest field suggesting the presence of a branched-chain sugar. Spin decoupling experiments revealed that this doublet was collapsed by irradiation at 3.27 ppm indicating that the adjacent proton is deshielded by an amine. In a similar manner evidence that the sugar is also 2-amino substituted was gained when irradiation at $\sim$3.4 ppm resulted in collapse of the anomeric proton doublet. This evidence of a 2,6-diamino-branched chain sugar combined with the observation of four methylene proton resonances between 1.8$\sim$2.7 ppm strongly suggests the presence of a 2,6-diamino-2,3,4,6-tetradeoxyheptapyranose, one of the purpurosamines which are commonly found in aminoglycoside antibiotics.

* Previously known as XK-70-1 and XK-70-A, respectively.

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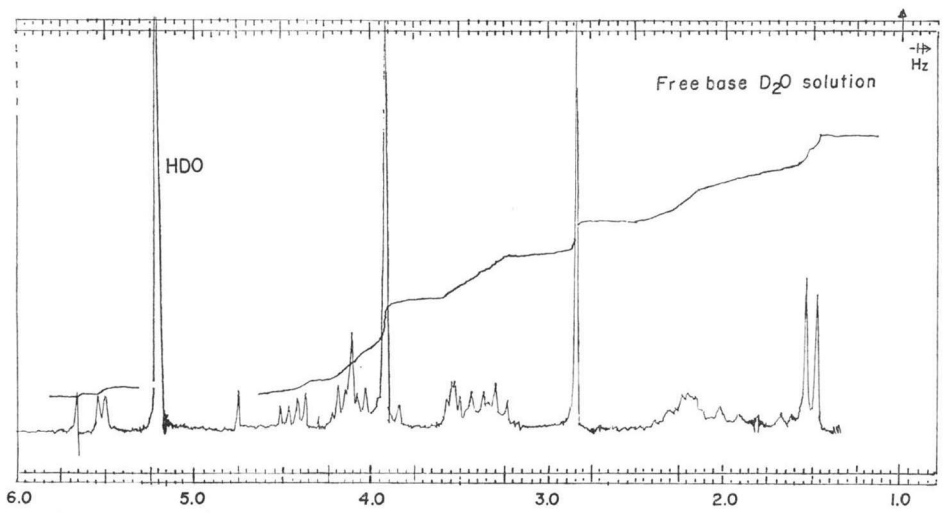
Fig. 1. 100 MHz PMR Spectrum of fortimicin B free base in D_2O.



This suggestion was confirmed by examination of the high resolution mass spectrum of fortimicin B free base (Table 2) which has a small but distinct molecular ion at *m/e* 348, shown by high resolution measurements to have the composition $C_{15}H_{32}N_4O_5$. As with many other aminoglycosides,[3] the molecular ion has a tendency to protonate, resulting in an $(M+1)^+$ ion of equal or greater intensity. In addition, other high molecular weight ions were observed arising from the elimination of small neutral fragments. However, the base peak

Table 1. 100 MHz PMR Parameters for fortimicin B free base and sulfate salt in D_2O solution

Chemical shifts

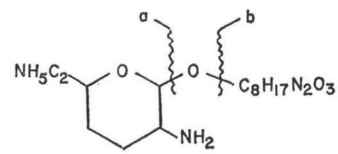
	Free base	Sulfate salt		Free base	Sulfate salt
H-1′	5.50	6.01	H-1	3.43	4.27
H-2′	~3.4	~4.0	H-2	4.17	5.08*
CH_2-3′,4′	1.8~2.7		H-3	4.11	4.54
H-5′	~4.0	~4.5	H-4	3.54	4.14
H-6′	3.27	3.85	H-5	4.44	5.04*
CH_3-6′	1.50	1.86	H-6	3.93	4.81
			OCH_3	3.92	4.02
			NCH_3	2.85	3.32

Coupling constants

	Free base	Sulfate salt		Free base	Sulfate salt
$J_{1',2'}$	3.8	3.5	$J_{1,2}$	9.5	4.5
$J_{5',6'}$	6.8	8.4	$J_{2,3}$	~3	3.5
			$J_{3,4}$	3.0	9.2
			$J_{4,5}$	4.5	3.2
			$J_{5,6}$	9.5	4.5
			$J_{1,6}$	9.5	4.5

Table 2. Significant mass spectral fragmentation of fortimicin B free base

m/e	% Relative intensity*	Formula+	Identification
349	0.5~2.0	$C_{15}H_{33}N_4O_5$	protonated molecular ion
348	0.5~1.5	$C_{15}H_{32}N_4O_5$	molecular ion
331	6	$C_{15}H_{29}N_3O_5$	$M^{+\cdot}-NH_3$
313	1	$C_{15}H_{27}N_3O_4$	$M^{+\cdot}-NH_3-H_2O$
305	1.5	$C_{13}H_{27}N_3O_5$	$M^{+\cdot}-C_2H_5N$
235	34	$C_9H_{19}N_2O_5$	$HO\diagdown H\rangle C=\overset{+}{O}$-*b*
217	6	$C_9H_{17}N_2O_4$	*m/e* 235$-H_2O$
207	81	$C_8H_{19}N_2O_4$	$H\diagdown H\rangle\overset{+}{O}$-*b*
189	16	$C_8H_{17}N_2O_3$	*b*
143	100	$C_7H_{15}N_2O$	*a*
126	26	$C_7H_{12}NO$	*a*$-NH_3$



* approximate values

\+ determined by high resolution mass measurements

Fig. 2. Details of ring proton resonances of fortimicin B free base.

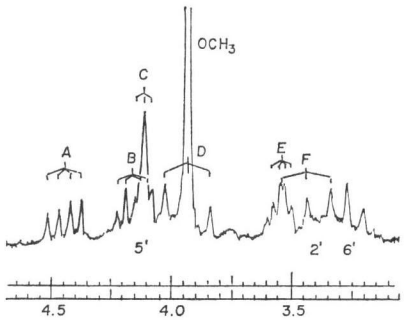


Fig. 3. Correlation of fortamine PMR and CMR

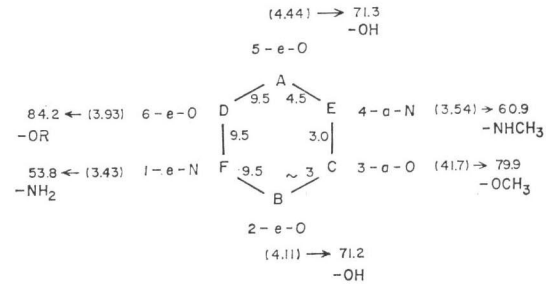


Table 3. 25 MHz CMR Parameters for fortimicin B free base in D_2O solution

	Purpurosamine of gentamine C_2	Fortimicin B pD 11.2		β-Shift
1′	102.9	102.5		6.5
		84.2	6	10.0
		79.9	3	5.8
5′	74.3	75.1		4.1
		71.3	5	5.8
		71.2	2	4.6
		60.9	4	
		59.3	OMe	
		53.8	1	
2′	50.8	50.6		
6′	50.0	50.4		
		35.4	NMe	
4′	26.0	27.4		
3′	27.0	27.1		5.6
7′	19.0	18.6		3.5

of the spectrum is *m/e* 143 ($C_7H_{15}N_2O$), an ion also prominent in the spectra of gentamicins C_2 and C_{2a}, which is attributed to a mono-methylated purpurosamine.[8] Comparison of the fortimicin B data with the spectra of these gentamicins suggests that the methyl substituent is located at either the C-6′ nitrogen or carbon. The presence of a *C*-methyl doublet in the PMR spectrum suggests purpurosamine B or a stereoisomer as the sugar moiety of fortimicin B. This observation then requires that the cyclitol portion (given the designation fortamine) must have a $C_8H_{17}N_2O_4$ molecular formula including both the *N*- and *O*-methyl groups. This was confirmed by the presence of fragments at *m/e* 235, 217, 207 and 189, a characteristic tetrad in cyclitol based fragmentations, resulting from sequential and combined losses of CO and H_2O. Each ion was supported by high resolution molecular formula (Table 2).

Additional details concerning the structure of fortamine were gained from a close examination of the ring proton resonances of the PMR spectrum of fortimicin B free base (Fig. 2). Spin-decoupling experiments showed that, in addition to H-2′, 5′ and 6′ associated with the sugar moiety, six resonances between 3.0 and 4.5 ppm (labeled $A \sim F$ in Fig. 2) were mutually coupled as summarized in Fig. 3. For example, proton *A* is coupled to both protons *D* and *E*. Since there are six low field protons each coupled to only two others, there can be no methylene group and the cyclitol must have a substituent at each position. The magnitudes of the couplings are also summarized in Fig. 3. The large couplings exhibited by protons *A*, *D*, *F* and *B* (for example, $J_{AD}=9.5$ Hz) require that they be axial with equatorial substituents (designated by *e* in Fig. 3) and the small couplings of the adjacent *C* and *E* protons require them to be equatorial with axial substituents (designated by *a* in Fig. 3). This arrangement of proton orientations establishes that fortamine has *chiro* stereochemistry with two adjacent axial substituents. The ring proton chemical shifts also summarized in parentheses in Fig. 3 segregate those

protons deshielded by nitrogen substituents (*E* and *F*) from those protons deshielded by oxygen substituents (*A*, *B*, *C* and *D*) and thereby further reveal the cyclitol to be an unusual 1,4-diamine. Fig. 3 also gives the numbering convention used for fortamine in which the equatorial nitrogen substituent is at position 1, the axial oxygen substituent at C-3 and the axial nitrogen substituent at C-4. Unfortunately, the PMR chemical shift differences are too small to differentiate between the various *N*- and *O*-substituents. CMR spectra were obtained for this purpose.

The 25 MHz CMR spectrum of fortimicin B free base in D_2O solution at pD 11.2 showed the required 15 resonances (Table 3). Seven of these resonances can be closely correlated with the published values of purpurosamine B in the pseudodisaccharide gentamine C_2.[4] It should be noted that, although it is possible to pair resonances between gentamine C_2 and fortimicin B, the differences in the chemical shifts, particularly that of C-5′, are greater than experimental error and may suggest some subtle difference exists between the two sugars.

Of the remaining eight resonances associated with fortamine, those at 35.4 and 59.3 ppm can be attributed to the *N*- and *O*-methyl groups by their quartet off-resonance single frequency decoupling (ORSFD) multiplicity, leaving six resonances to be assigned to the cyclitol ring carbons. Extensive single frequency decoupling (SFD) experiments were performed to relate specific proton resonances with the carbon to which they are attached as summarized in Fig. 3. For example, the furthest downfield proton at 4.44 ppm (H-5) is attached to the carbon having a 71.3 ppm chemical shift. Several publications have appeared in which CMR spectra of many aminoglycosides have been extensively interpreted.[4~10] These published values can be used to generate chemical shift ranges for specific structural types which are collected in Table 4.

In this manner, the two resonances for C-1 and C-4 at 53.8 and 60.9 ppm can be confirmed to be deshielded by nitrogen, and furthermore, that the resonance at lower field attributed to C-4 is influenced by the β-shift of the *N*-methyl group. Two carbons with nearly identical chemical shifts, C-2 and C-5, (71.3, 71.2 ppm) fall in the range of those deshielded by hydroxyl groups. The lowest field resonance at 84.2 ppm is in the characteristic range of those carbons deshielded by a glycosidic linkage, thereby specifying C-6 as the point of sugar attachment. The remaining resonance of C-3 at 79.9 ppm is outside the range for hydroxyl substitution and is therefore the site of methoxyl substitution deshielded by the β-shift of the *O*-methyl group. Consistent with the proposal that fortamine is a 1,4-diamine is the observation that each of the oxygen substituted carbons show substantial β-protonation shifts (Table 3). Characteristically the largest β-shift is associated with the site of sugar attachment. The resulting pattern of substituents is summarized in Fig. 3 and when combined with the identification of the sugar as a branched diamine defines the structure of fortimicin B to be **1**.

Table 4. Typical CMR chemical shifts Obtained from published aminoglycoside data. Quoted downfield from TMS, measure from dioxane=67.4 ppm.

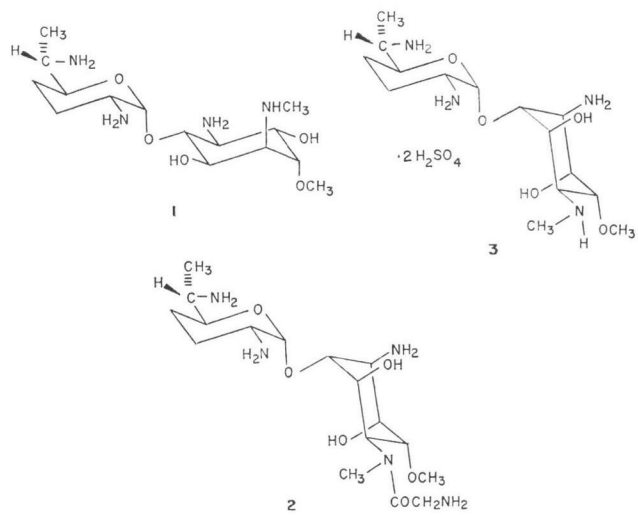
Deshielding influence	Carbon type		
	CH	CH_2	CH_3
$C\text{-}O_2$	109.1~98.8	—	—
C-OH*	78.8~64.2	68.7~60.8	58.7~55.6
C-O-Sugar	89.0~83.0	—	—
$C\text{-}OCH_3$	80.2	—	—
$C\text{-}NH_2$	57.7~49.6	46.1~42.0	38.2~33.6
$C\text{-}NHCH_3$	64.7~58.0	55.1	—
C-C	—	37.4~26.0	23.0~15.0

One can generally differentiate carbon type from off-resonance single frequency decoupling experiments where CH become doublets, CH_2 triplets and CH_3 quartets.

* includes C-5 in hexoses and pentoses.

Fortimicin A

The molecular ion of fortimicin A appears at *m/e* 405 along with the protonated species of about equal intensity at *m/e* 406 (Table 5). Exact mass measurements indicate an empirical formula of $C_{17}H_{35}N_5O_6$ for the molecule. Initial losses in the high mass range previously seen in the spectra of fortimicin B free base are also present; however, in each case, they are shifted by 57 amu (C_2H_3NO). Evidence for the existence of a branched diaminosugar exists in the presence of strong ions at *m/e* 143 ($C_7H_{15}N_2O$) and *m/e* 126 ($C_7H_{12}NO$) which are also prominent in the spectrum of fortimicin B. The increase in molecular weight is shown to be localized with the cyclitol as evidenced by the shift of the tetrad to *m/e* 292, 274, 264 and 246 (Table 5). The possibility suggested by this data that fortimicin A is identical to fortimicin B with an additional C_2H_4NO group attached to fortamine was confirmed by chemical manipulations reported in the preceding paper[2] and Scheme 1.

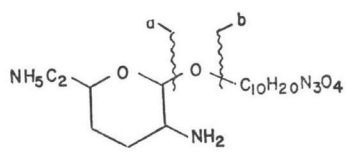


Most significant was the observation that barium hydroxide hydrolysis of fortimicin A gave fortimicin B, thereby proving the suspected identity of the diaminosugar and the cyclitol in the two compounds. Under vigorous acidic hydrolysis fortimicin A gives a mixture of hydrolysis products which by tlc are completely mirrored by those of fortimicin B with the exception of a single component.[2] This additional component was shown to be glycine by tlc comparison of the dansyl derivative prepared from the hydrolysis mixture of fortimicin A and from an authentic reference. The same dansyl derivative could also be generated by derivatization of intact fortimicin A followed by hydrolysis indicating that the amino group of glycine is free in the intact antibiotic. The point of attachment of the glycine to the cyclitol moiety was disclosed by examination of the 100 MHz PMR data of fortimicin A free base.

The most significant difference between the 100 MHz PMR spectrum of fortimicin A free base in D_2O (Table 6) and fortimicin B free base

Table 5. Significant mass spectral fragmentation of fortimicin A free base

m/e	% Relative intensity*	Formula	Assignment
406	0.5~1.5	$C_{17}H_{36}N_5O_6$	$(M+H)^+$
405	0.7~2.0	$C_{17}H_{35}N_5O_6$	molecular ion
388	4	$C_{17}H_{32}N_4O_6$	$M^{+\cdot}-NH_3$
370	1	$C_{17}H_{30}N_4O_5$	$M^{+\cdot}-NH_3-H_2O$
362	1.5	$C_{15}H_{30}N_4O_6$	$M^{+\cdot}-C_2H_5N$
292	13	$C_{11}H_{22}N_3O_6$	$HO\backslash H\rangle C=\overset{+}{O}$-*b*
274	24	$C_{11}H_{20}N_3O_5$	*m/e* 292 $-H_2O$
264	14	$C_{10}H_{22}N_3O_5$	$H\backslash H\rangle \overset{+}{O}$-*b*
246	82	$C_{10}H_{20}N_3O_4$	*b*
143	100	$C_7H_{15}N_2O$	*a*
126	23	$C_7H_{12}NO$	*a* $-NH_3$



* Approximate values

Fig. 4. Details of ring proton resonances of fortimicin A free base in D_2O.

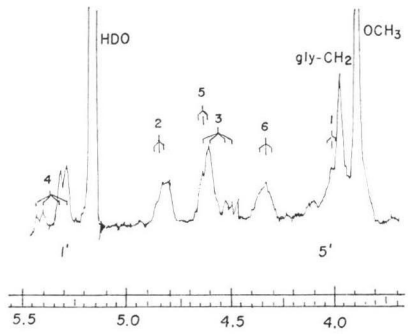
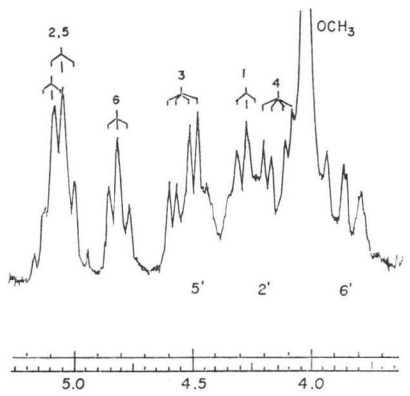


Fig. 5. Details of ring proton resonances of fortimicin B sulfate in D_2O.



Scheme 1. Hydrolysis and identification of glycine.

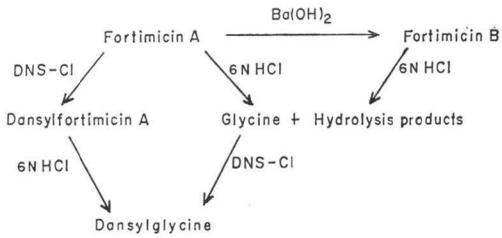


Table 6. 100 MHz PMR Parameters of fortimicin A free base and sulfate in D_2O solution

Chemical shifts

	Free base	sulfate		Free base	sulfate
H-1′	5.30	5.80	H-1	~4.0	4.34
H-2′	~3.3	~4.0	H-2	4.83	5.16
CH_2-3′, 4′	1.8~2.4	1.8~2.7	H-3	4.56	~4.7
			H-4	5.36	5.36
H-5′	~4.0	~4.7	H-5	4.63	~4.7
H-6′	3.31	3.83	H-6	4.33	~4.4
CH_3-6′	1.51	1.80	O-CH_3	3.89	3.96
gly-CH_2	3.97	4.54	N-CH_3	3.50	3.59

Coupling constants

	Free base	Sulfate		Free base	Sulfate
$J_{1',2'}$	3.0	3.0	$J_{1,2}$	~3	~3
$J_{5',6'}$	6.5	6.5	$J_{2,3}$	3.0	~3
			$J_{3,4}$	~11	10.0
			$J_{4,5}$	3.0	2.0
			$J_{5,6}$	~3	~3
			$J_{6,1}$	3.0	~3

measured under identical conditions (Table 1) is the marked deshielding of the *N*-methyl resonance in the former to 3.50 ppm from 2.85 ppm in the latter. This is interpreted to be a consequence of conversion of the 4-nitrogen from amine to amide and thereby supports fortimicin A as the 4-*N*-glycine amide of fortimicin B. Further scrutiny of the PMR spectrum of the free base as shown in Fig. 4 reveals an additional and interesting feature of the structure of fortimicin A.

The resonance of H-4 deshielded by the presence of an amide now exhibits one large coupling (J=11 Hz) of a magnitude sufficient to require it to have an axial orientation. Spin-decoupling experiments reveal that this is the only large coupling exhibited by the cyclitol ring protons (Table 6). This requires that the fortamine ring has inverted from the $^4C_1$ conformation as shown for fortimicin B (**1**) to the $^1C_4$ conformer which incorporates four axial substituents in fortimicin A (**2**).

This unusual but not unprecedented[11] conformational inversion was shown not to be limited to fortimicin A when the 100 MHz PMR spectrum of fortimicin B sulfate in D_2O was obtained (Fig. 5, Table 1). The coupling constants reveal the same pattern with $J_{3,4}$ being the only large coupling requiring ring inversion

in this compound as shown in **3**. It should be stated that both in the case of fortimicin A (**2**) and fortimicin B sulfate (**3**), the magnitude of $J_{3,4}$ is sufficiently large to require a true diaxial relationship (180° dihedral angle) between H-3 and H-4. However, the magnitudes of the remaining couplings, although small, do not rule out the presence of significant populations of numerous possible flexible non-chair conformation in the remainder of the ring in which the substituents may not be truly axial. The representation given for the structures in **2** and **3** is used for convenience and is not meant to specify a rigid ring. Alternatively, however, in the case of fortimicin B free base (**1**) the magnitudes of all the ring proton coupling constants define a regular $^4C_1$-chair conformation.

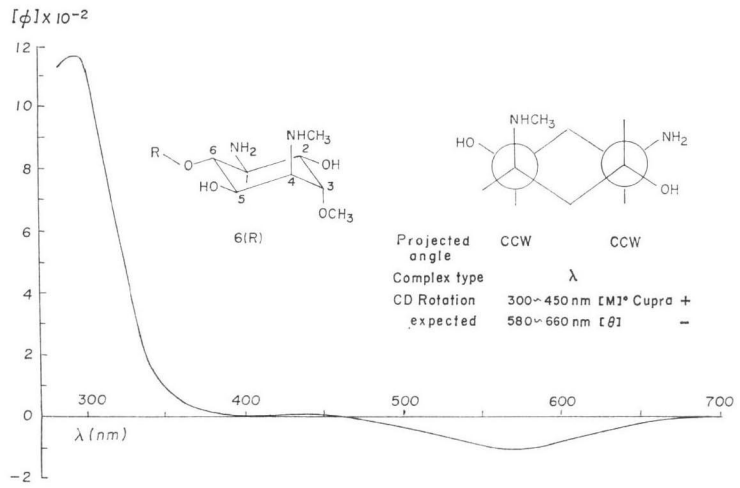
Table 7. 25 MHz CMR Parameters of fortimicin A in D_2O solution

	Fortimicin B sulfate	Fortimicin A sulfate	Fortimicin A free base	β-shift
C-1′	96.0	95.4	100.1	4.7
C-2′	51.9	51.7	50.5	
C-3′	21.5	21.6	26.9	5.3
C-4′	26.3	26.3	27.3	
C-5′	71.0	70.9	74.9	4.0
C-6′	49.4	49.4	50.1	
6′-CH_3	15.1	15.0	18.4	3.4
C-1	53.8	54.1	52.5	
C-2	66.3	66.3	71.1	4.8
C-3	74.5	72.4	72.9	
C-4	58.0	51.8	55.4	
C-5	66.8	71.6	73.7	
C-6	74.3	74.5	78.4	3.9
O-CH_3	57.9	56.8	56.4	
N-CH_3	32.4	32.0	32.2	
gly CH_2	—	41.3	43.3	
gly CO	—	168.8	176.2	

Finally, the 25 MHz CMR spectrum of fortimicin A sulfate in D_2O is summarized in Table 7 and compared with that of fortimicin B sulfate also obtained at pD 1.8. The correspondence of diaminosugar chemical shifts is as expected with the only significant difference exhibited by C-1 attributed to conformational effects about the glycoside bond. An additional low-field carbonyl resonance and a 41.3 ppm resonance shown by ORSFD to be a triplet confirm the presence of glycine. Although the substituent effects exhibited by the fortamine resonances are irregular, the loss of two β-protonation shifts by C-3 and C-5 confirms the location of glycine at N-4.

The previous considerations have defined the structures of fortimicins A and B in terms of their relative stereochemistry. Absolute configurations of both the diaminosugar and fortamine were obtained

Fig. 6. CD spectrum for fortimicin B in Cupra A solution.

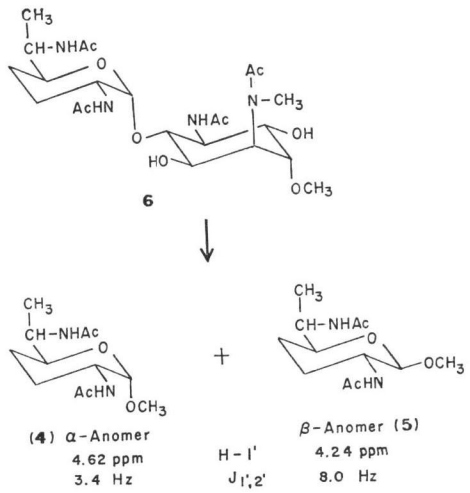


using classical chemical and optical methods.

Absolute Configuration of Fortamine

The CD spectrum of fortimicin B free base in Cupra A solution[12] (Fig. 6) exhibits an intense positive rotation at short wave lengths with a less intense negative rotation at longer wave lengths characteristic of a λ-complex.[13] The complex must arise from the vicinal aminoalcohols in the cyclitol since the purpurosamine is inert to the reagent. This type of complex is formed when the front to back angle described by the complexing groups is counterclockwise (Fig. 6). As shown in the projection formula, the two sites for complexation in fortamine have the same reinforcing counterclockwise (CCW) angle in the 6R stereoisomer, thereby defining its absolute configuration. It is interesting to note that the centers in the C-1, 5, 6 ring segment including the position of glycosidation have the same absolute stereochemistry as the analogous centers in the C-3, 4, 5 ring segment of 2-deoxystreptamine[14,15] in the gentamicins including the position of glycosidation.

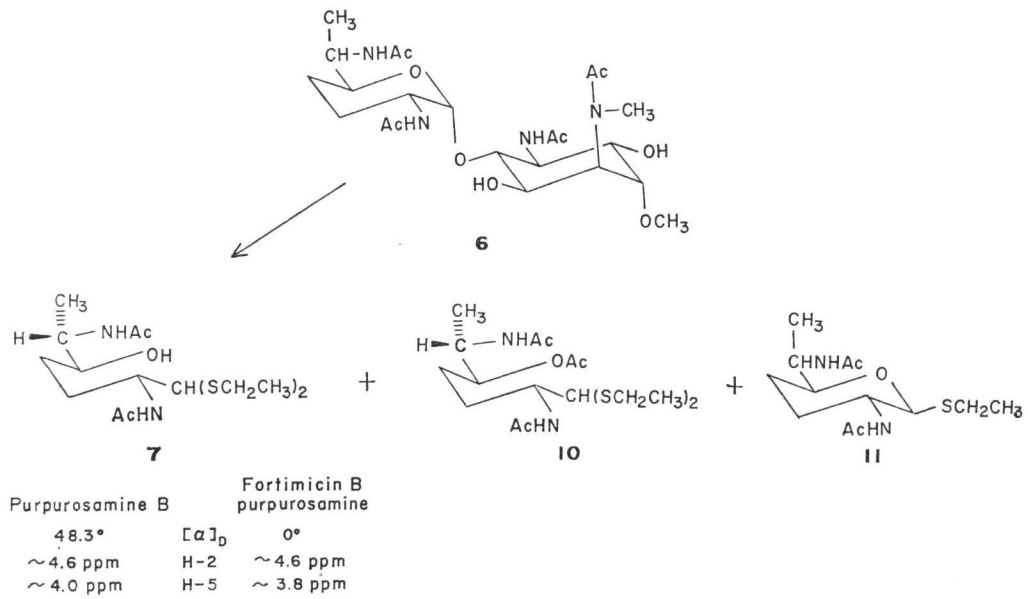
Scheme 2. Preparation and characterization of methyl glycosides.



Absolute Configulation of the Diaminosugar

The absolute configuration of the diaminosugar of the fortimicins was determined by its relationship to purpurosamine B. This was accomplished by application of the degradation schemes used by the Schering workers.[16,17]

Scheme 3. Mercaptolysis products.



That purpurosamine B is a D-sugar was established by application of HUDSON's rule of optical rotation to the anomeric methyl glycosides of 2,6-di-*N*-acetylpurpurosamine B which showed the difference $[M]_\alpha - [M]_\beta$ to be $+442°$. The corresponding di-*N*-acetyl methyl glycosides (**4** and **5**) of the fortimicin diaminosugar were prepared by methanolysis of tetra-*N*-acetylfortimicin B (**6**) followed by re-*N*-acetylation (Scheme 2). The anomers **4** and **5** were characterized by the chemical shift and coupling constants exhibited by their 100 MHz PMR spectra (Scheme 2). Confirmation that the C-5 1-acetamidoethyl substituents of **4** and **5** have equatorial orientations was derived from proton decoupling experiments carried out on the α-anomer **4** in which the orientation of H-5 was shown to be axial by the magnitude of the sum of its vicinal couplings (Scheme 2). In a similar manner the large magnitude of the anomeric coupling of the β-anomer **5** confirms the equatorial orientation of the 2-acetamide group. The molecular rotation difference $[M]_\alpha - [M]_\beta = +429°$ for **4** and **5** established that the diaminosugar of the fortimicins is also a D-sugar. Since the above relationships establish that the diaminosugar and purpurosamine B have the same configurations at C-2 and C-5, the only remaining center to be compared is C-6.

Since the Schering group established the C-6 configuration of purpurosamine B from its di-*N*-acetyl diethyldithioacetal[16,17], a similar procedure was followed for the fortimicins (Scheme 3).

Mercaptolysis of tetra-*N*-acetylfortimicin B (**6**) followed by re-*N*-acetylation gave a di-*N*-acetyl diethyldithioacetal (**7**). The mass spectrum of **7** showed a molecular ion and fragment peaks identical with those reported[17] for di-*N*-acetyl diethyldithioacetal of purpurosamine B. The 100 MHz pyridine-d_5 solution spectrum at 110°C of **7** was very similar to that published[17] for the purpurosamine B derivative but showed a significant difference in the chemical shift of H-5 (Scheme 3) suggesting a possible configurational difference at C-6. That the diaminosugar of fortimicin is diastereomeric to purpurosamine B was clearly established by the marked differences in the optical rotations of the corresponding di-*N*-acetyl diethyldithioacetals (Scheme 3). The positive optical rotation reported for the derivative of purpurosamine B was not observed although an intense negative COTTON effect was evident at 245 nm. Cognizant of the results from the methyl glycosides **4** and **5**, these data indicate that the diaminosugar of the fortimicins is 6-*epi*-purpurosamine B. This same epimeric sugar has recently also been reported as a constituent of a gentamicin minor component.[18]

Summary

This then concludes the structure elucidation of fortimicin B and fortimicin A whose total structures are given as **1** and **2** respectively. The structures so defined are novel in the aminoglycoside class of antibiotics and incorporate several unusual features: *chiro* cyclitol stereochemistry, 1,4-diamino substitution of the cyclitol, conformationally flexible cyclitol, presence of a simple amino acid and 6-*epi*-purpurosamine B.

Experimental

Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 50 eV using the direct insertion probe. High resolution data was acquired on a PDP-11 computer. PMR 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 a co-axial capillary in the sample tube. Conversion to the commonly applied internal TSP scale in D_2O can be made by δ TMS (external) = δTPS (internal) + 0.42 ppm. CMR spectra were measured on a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm

downfield from TMS. Optical rotations were measured with a Hilger and Watts polarimeter. CD spectra were obtained at 29°C (cell compartment temperature) in a Durrum-Jasco ORD/UV/CD 5 instrument. Reported pD values are uncorrected pH meter readings of deuterated solutions.

Hydrolysis of Fortimicin A (**2**) to Fortimicin B (**1**)

A mixture of 303.3 mg of fortimicin A disulfate, 7.61 g of $Ba(OH)_2 \cdot 8\ H_2O$ and 24 ml of deionized water was heated in an oil bath at 100°C for 3.5 hours with magnetic stirring. The resulting mixture was allowed to stand overnight and then filtered through a Celite mat. Carbon dioxide was bubbled through the filtrate and the resulting $BaCO_3$ was removed by filtration. The carbon dioxide treatment was repeated, the filtrate was concentrated under reduced pressure, and the residue was passed through a small column containing 5 ml of AG2-X8 (OH) resin. Elution with water gave 58 mg of fortimicin B, identical in all respects with the product obtained from the fermentation.

Detection of Glycine

A 1.0-mg sample of fortimicin A sulfate was hydrolyzed in 2 ml of 6 N HCl in a sealed tube for 17 hours at 110°C. The hydrolysate was evaporated under vacuum several times from water to remove excess HCl. The residue was resuspended in 0.25 ml H_2O. A 3-μl portion of this mixture was treated with 10 μl of 0.1 M $NaHCO_3$ and 10 μl of a dansyl chloride reagent (1.0 mg/ml in acetone). The mixture was allowed to stand 2 hours at 23°C in the dark, then mixed with 5~10 volumes of 2% triethylamine in 10% ethanol in water. The solution was evaporated to dryness and residues resuspended in 0.5 ml ethanol = solution A.

A 1.5-mg/ml solution of glycine was prepared in water. A 1-μl portion of this solution was treated with 10 μl of 0.1 M $NaHCO_3$ and 10 μl of dansyl chloride reagent (1 mg/ml in acetone) and processed exactly as previous = solution B.

A solution of fortimicin A sulfate was mixed with 10 μl of 0.1 M $NaHCO_3$ and 10 μl of dansyl chloride reagent (1 mg/ml in acetone). The mixture was allowed to react for 2 hours in the dark and concentrated to a residue which was suspended in 0.5 ml of 6 N HCl. Hydrolysis was accomplished in a sealed tube at 110°C for 4 hours. The hydrolysate was concentrated several times from water to remove excess HCl. The residue was dissolved in 0.2 ml ethanol = solution C.

Solutions A, B and C were each simultaneously spotted on Quantagram silica gel plates and co-chromatographed in $CHCl_3$ - MeOH -glacial acetic acid (75: 20: 5). The plates were visualized by UV light at 360 nm and in each case only two spots were observed: DNS-OH Rf = 0.54 and DNS-glycine Rf = 0.715.

1,4,2′,6′-Tetra-*N*-acetylfortimicin B (**6**)

A solution of fortimicin B (3.92 g) in 270 ml absolute methanol was cooled to 0°C in an ice-bath and treated dropwise with 14 ml of acetic anhydride. After stirring for 0.5 hour, an additional 14 ml of acetic anhydride was added dropwise. Stirring was continued at 0°C for 0.5 hour and then at room temperature for 4 hours. The solution was evaporated under reduced pressure and co-distilled with benzene, then methanol and finally again with benzene to give the acetate **6** (6.15 g) as a white powder: $[\alpha]_D + 92.72°$ (*c* 1.0, MeOH); IR: 1630, 1520 cm^{-1}; Mass Spec: $M^{+\cdot}$ obsd $C_{23}H_{40}N_4O_9$ requires 516.2795, measd, 516.2763; PMR spectrum (D_2O): 1.53, d, 6′-CH_3; 2.39, 2.42, 2.45, 2.46, 2.57, s, *N*-$COCH_3$ rotamers; 3.43, 3.56, s, *N*-CH_3 rotamers; 3.85, 3.86, s, *O*-CH_3 rotamers.

2,6-Di-*N*-acetyl-6-*epi*-purpurosamine B α- and β-methyl glycosides (**4** and **5**)

A solution of 6.15 g of tetra-*N*-acetylfortimicin B and 400 ml of 6 N HCl in dry methanol was heated under reflux for eight hours and then allowed to stand overnight at room temperature. Evaporation of the solvent under reduced pressure left 7.44 g of brown glass. The glass was dissolved in water and passed through a column of AGl-X8 (OH) resin. The basic eluates were taken to dryness under reduced pressure and co-evaporated with ethanol to remove residual water.

To a magnetically stirred solution of the methanolysis product in 270 ml of methanol, cooled to 0°C in an ice-bath, was added 14 ml of acetic anhydride. Stirring was continued at 0°C for 0.5 hour and then an additional 14 ml of acetic anhydride was added. Stirring was continued at 0°C for 0.5 hour and then at room temperature for 24 hours. The methanol was evaporated under reduced pressure and the acetic anhydride was removed by co-distillation with benzene and methanol to yield 5.94 g of

a pale yellow glass.

A portion of the glass (1.5 g) was chromatographed on a column (2.1 × 43 cm) of silica gel prepared and eluted with $CHCl_3$ - MeOH (8.5: 1.5, v/v). Initial fractions gave 388 mg of methyl 2,6-di-*N*-acetyl-6-*epi*-α-D-purpurosaminide B (**4**) which crystallized from methanol, mp 217~218°C, $[\alpha]_D^{24}$ + 55.2° (*c* 0.97, CH_3OH); IR: 3303, 3282, 3100, 1641, 1556 cm^{-1}; Mass Spec: $M+1^{+\cdot}$ 259; Anal: Calcd. for $C_{12}H_{22}N_2O_4$, C, 55.80; H, 8.59; N, 10.84; Found: C, 56.02; H, 8.87; N, 10.68; PMR spectrum ($CDCl_3$): 1.19, d, 6′-CH_3; 1.98, 2.00, s, *N*-$COCH_3$; 3.37, s, *O*-CH_3.

Continued elution gave 171 mg of methyl 2,6-di-*N*-acetyl-6-*epi*-β-D-purpurosaminide B which crystallized from methanol, mp. 210~211°C; $[\alpha]_D^{24}$ −111° (*c* 0.94, CH_3OH); IR: 3286, 3100, 1642, 1551 cm^{-1}; Mass Spec: $M+1^{+\cdot}$ 259; Anal: Calcd for $C_{12}H_{22}N_2O_4$, C, 55.80; H, 8.59, N, 10.84; Found: C, 55.04; H, 8.63; N, 10.58; PMR spectrum ($CDCl_3$): 1.22, d, 6′-CH_3; 1.98, 2.00, s, *N*-$COCH_3$; 3.47, s, *O*-CH_3.

2,6-Di-*N*-acetyl-6-*epi*-purpurosamine B diethyldithioacetal (**7**)

1,4,2′,6′-Tetra-*N*-acetylfortimicin B (**6**, 838 mg) was treated with a mixture of cold ethanethiol (2.5 ml) and concentrated hydrochloric acid (2.5 ml) and stirred at room temperature for 22 hours. Excess reagent was removed by evaporation under vacuum by heating on a steam bath for 5 minutes followed by 30 minutes at room temperature. The residue was twice dissolved in 50-ml portions of water and taken to dryness. After neutralization with AG2-X8 (OH form) and drying, the solid was dissolved in 45 ml of methanol and cooled in an ice-bath. The stirred solution was treated with acetic anhydride (5 ml). Stirring was continued at 4°C for 0.5 hour, then at room temperature for 23 hours. The methanol was evaporated under vacuum and residual acetic anhydride removed by co-evaporation with benzene. The resulting solid (943 mg) was chromatographed on a column (2.2 × 49 cm) of silica gel using the solvent system methylene chloride- 95% aqueous methanol - concentrated ammonium hydroxide (18:2:0.2). Column effluents were monitored by TLC. Initial fractions gave the triacetate **10** followed by fractions containing **11**. Later fractions gave pure *N*-acetyl-6-*epi*-purpurosamine B diethyldithioacetal (**7**) followed by fractions contaminated with other products. The contaminated fractions were rechromatographed with the same system to give a total of 97 mg of the dithioacetal 7: $[\alpha]_D^{24}$—2.9° (*c* 1.0, MeOH); Anal: Calcd. for $C_{15}H_{30}N_2S_2O_3$, C, 51.40; H, 8.63; N, 7.99; Found: C, 51.35; H, 8.48; N, 7.82; IR: 1650, 1512 cm^{-1}; MS: $M^{+\cdot}$ measured 350.1706; $C_{15}H_{30}N_2S_2O_3$ requires 350.1698; PMR spectrum (pyridine-d_5): 1.20, 1.25, t, CH_3; 1.31, d, 6′-CH_3; 2.06, 2.11, s, *N*-$COCH_3$.

5-*O*-Acetyl-2,6-di-*N*-acetyl-6-*epi*-purpurosamine B diethyldithioacetal (**10**)

PMR spectrum ($CDCl_3$): 1.13, d, 6′-CH_3; 1.25, 1.29, t, CH_3; 2.00, 2.03, 2.08, s, *N* and *O*-$COCH_3$.

Ethyl 2,6-di-*N*-acetyl-1-thio-β-6-*epi*-purpurosamine B (**11**)

PMR spectrum ($CDCl_3$): 1.15, d, 6′-CH_3; 1.29, t, CH_3; 1.88, 1.89, s, *N*-$COCH_3$.

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