Structure of the Major Glycopeptide of the Teicoplanin Complex

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Abstract: The structure of the pseudoaglycon of the teicoplanin glycopeptides is described. The ψ-aglycon has a peptide core that is identical with antibiotic A41030B, and *N*-acetylglucosamine is attached to the aglycon through the benzylic hydroxyl group at the A-1′ position. The teicoplanin glycopeptides have a mannose moiety linked to the ψ-aglycon through a phenol at D-5 and an *N*-acylglucosamine attached through the B-4 phenol; the acyl chain is variable, giving rise to the various members of the teicoplanin complex. The major teicoplanin glycopeptide has the composition $C_{88}H_{97}N_9O_{33}Cl_2$ (integral molecular weight = 1877) and the structure **11**, where acyl = $C_{10}H_{19}O$ (isodecanoyl). The structure elucidation is based on ^1H NMR studies, FAB/MS studies, and the results of amino acid analysis.

Teicoplanin (teichomycin) is a complex of closely related antibiotics produced by *Actinoplanes teichomyceticus*;[1] the teicoplanin factors (formerly designated as teichomycin A_2) are glycopeptides with properties similar to vancomycin and related antibiotics.[2] Teicoplanin acts by blocking cell wall biosynthesis,[3] as do the other members of the glycopeptide class,[4] and it has a spectrum of activity similar to that of vancomycin.[1,5] Teicoplanin is more antimicrobially active than vancomycin in vitro,[5] and it exhibits substantially different pharmacokinetic behavior in vivo, having a half-life of 40 h in man.[6] We have determined the teicoplanin structure as a first step toward understanding the unusual pharmacological features of this antibiotic; the structure elucidation for the major glycopeptide of the teicoplanin complex is described in this paper. This structure has also been determined independently in the laboratory of Prof. D. H. Williams.[7] Teicoplanin is the first glycopeptide antibiotic that contains glucosamine rather than a 2,3,6-trideoxy-3-amino sugar,[2] but the most unusual structural feature of this complex is the presence of an acyl chain; variations in the acyl moiety give rise to the various members of the teicoplanin complex.

A typical glycopeptide antibiotic contains a peptide core of complex amino acids, one or more neutral sugar moieties, and one or more amino sugars. Proton NMR spectroscopy is an extremely useful tool in the structure elucidation of such materials, but the ^1H NMR spectrum of a glycopeptide complex is often difficult to interpret for two reasons: (a) the spectrum of a complex will usually be broadened due to the heterogeneity of the sample (the various members of a complex sometimes differ only in the nature or distribution of the groups attached to the peptide core),[8] and (b) the spectrum of even a single glycopeptide species will often be broadened by apparent aggregation in solution. Both of these difficulties are conveniently eliminated (if the members of a complex share a common aglycon) by removal of phenolicly linked sugars by acid hydrolysis;[9] the resulting ψ-aglycon (peptide core plus a single amino sugar) is identical for each member of the complex, and the tendencies toward aggregation are greatly reduced after reduction of the sugar content. The NMR spectra of ψ-aglycons are more readily interpretable than those of the parent antibiotics in such cases, and a convenient approach to glycopeptide structure elucidation has been to first determine the structure of the ψ-aglycon followed by study of how the remaining portions of the parent molecule are attached to the peptide core.[8-12] This is the approach employed in the study of teicoplanin described in this report.

Experimental Section

Teicoplanin complex (teichomycin A_2) was received from Gruppo Lepetit (Milan, Italy). The complex was also isolated following fermentation of *Actinoplanes teichomyceticus*, ATCC 31121; the two materials were equivalent. The major factor of the teicoplanin complex was obtained after further purification; physical characterization of the isolated major factor gave results identical with those reported here.

Preparation of Teicoplanin Pseudoaglycon (ψ-Aglycon) Methyl Ester. Teicoplanin complex (30 mg) was dissolved in 3% HCl in methanol (10 mL) and heated to reflux for 2 h. The reaction mixture was cooled, concentrated to dryness in vacuo, and treated with 1 mL of water plus 5 mL of acetonitrile. A solid was isolated by filtration of the resulting mixture; the filtrate was also saved for further analysis. The solid was reprecipitated from methanol/acetonitrile and dried in vacuo, yielding 12 mg of ψ-aglycon. Thin-layer chromatography [$CHCl_3/CH_3OH/NH_4OH$ (2:3:1), on silica gel] one major spot, R_f 0.28; R_f for teicoplanin complex in this system is 0.06.

An aliquot of the filtrate obtained at the first precipitation of the ψ-aglycon was examined by amino acid analysis without further hydrolysis; no free amino groups were detected. A second sample of the filtrate was analyzed after hydrolysis with HCl; the resulting chromatogram contained a peak identified as glucosamine by comparison with an authentic sample.

FAB Mass Spectrometry. Fast atom bombardment mass spectra were obtained on a ZAB-3F mass spectrometer using 8 keV xenon atom bombardment. The samples were dispersed in a mixture of glycerol, methanol, and oxalic acid. For cationization an approximately equal volume of glycerol containing 0.25 M potassium chloride was added to the dispersed sample.

NMR Spectroscopy. Proton NMR spectra were recorded at 60 °C or at ambient temperature (~23 °C), using a Bruker WH360 spectrometer in the Fourier transform mode. Solutions were prepared in either Me_2SO-d_6 or D_2O. Nuclear Overhauser effect (NOE) measurements were performed under nonoptimized conditions, as described previously;[10,11] NOE values reported in the text have only qualitative significance.

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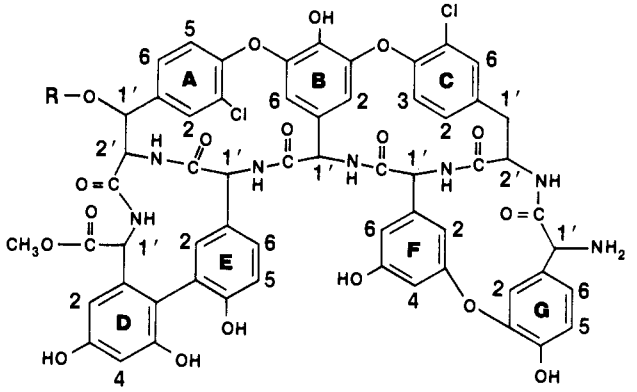
Amino Acid Analysis. Samples were either hydrolyzed with 6 N HCl for 21 h or were analyzed directly without hydrolysis. Amino acid analyses were carried out on a modified Beckman 120B automated analyzer.

Results and Discussion

The 360-MHz ^{1}H NMR spectrum of the teicoplanin methanolic hydrolysis product has been assigned. The assignments, which indicate that the product has the structure **1**, are based on homonuclear decoupling studies, NOE observations, and comparison with data for other glycopeptides. The spectrum of **1** contains a three-proton singlet near 3.7 ppm, as do the spectra of the ψ-aglycons of actaplanin[11] and A35512B;[10] the peak indicates that the peptide core has a methyl ester at its carboxy terminal. However, this resonance is not present in the spectrum of the intact teicoplanin glycopeptides; the free carboxyl group of the parent antibiotic aglycon has been esterified during the hydrolysis in methanol to produce the teicoplanin ψ-aglycon methyl ester (**1**).



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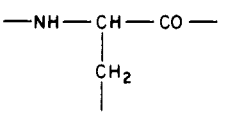
Amino Sugar of 1. Teicoplanin contains D-mannose and D-glucosamine as its carbohydrate components,[2] and the presence of glucosamine in **1** has been verified by amino acid analysis. [The amino acid analysis chromatogram of **1** contains a peak for ammonia, four peaks that are similar to those found for antibiotic A41030B (see below),[13] and an additional peak having a retention time of 9995 s; the retention time for D-glucosamine hydrochloride in the same system is 9992 s.]

Typical glycopeptide antibiotics have six amide bonds and one amino terminal group on their aglycon,[4] but seven amide linkages have been identified in the NMR spectrum of **1**, using spin decoupling results. Six of the seven peptide bonds are listed in Table I; they will be discussed later in this report. The "extra" amide NH (7.74 ppm, $J \sim 8$ Hz) is coupled to an overlapped resonance at 3.35 ppm, which is in turn coupled to a doublet at 4.35 ppm ($J \sim 8$ Hz). These connections indicate that the nitrogen involved in the amide bond is not the peptide amino terminal group attached at G-1′ of **1**; the new amide bond must involve the nitrogen of glucosamine. The presence of a three-proton singlet at 1.86 ppm in the NMR spectrum of **1** (and also in the spectrum of the parent glycopeptide) indicates that the amino sugar of the teicoplanin ψ-aglycon is *N*-acetylglucosamine. Both the relatively high-field position of the anomeric proton resonance (4.35 ppm) and the large value of $J_{1,2}$ (~8 Hz) suggest that the *N*-acetylglucosamine is linked to the teicoplanin aglycon as the β anomer; teicoplanin differs in this respect from ristocetin,[12] avoparcin,[15] A35512B,[10] and actaplanin,[11] all of which have amino sugars attached to their peptide cores as α anomers.

Peptide Core of 1. Nonsugar resonances in the proton NMR spectrum of the teicoplanin ψ-aglycon ester (**1**) are listed in Table I; the resonances were sorted into substructure groups (aromatic rings, amide linkages, etc.) through the use of spin–spin decoupling experiments and nuclear Overhauser effect (NOE) observations.

One of the six amide resonances of the aglycon (8.12 ppm) is coupled to a CH (4.94 ppm), which is also coupled to a CH_2 (3.33 and 2.88 ppm); such a fragment, **2**, is also found in the actaplanins[11] and the A41030 antibiotics.[13] Irradiation of the broadened doublet at 2.88 ppm produces a negative NOE for a partially overlapped aromatic doublet at 7.82 ppm; a similar NOE is observed in actaplanin ψ-aglycon (irradiating the C-1′ resonance at 2.88 ppm causes a 30% reduction of the actaplanin C-2 doublet at 7.85 ppm).[11] The similarities in both chemical shifts and NOEs (see Tables I and II) suggest that in the teicoplanin aglycon, as in actaplanin, **2** is attached to the aromatic ring labeled C (in **1**). The other five amide resonances occur in four (NH–CH) pairs, plus one larger fragment, **3**; the group R in **3** is the ψ-aglycon amino sugar, *N*-acetylglucosamine. The **3** NH (6.02 ppm) is coupled to a doublet at 4.11 ppm which sharpens slightly when a singlet at 5.27 ppm is irradiated; the 4.11 and 5.27 ppm resonances are both involved in a number of NOE observations which suggest that **3** is attached to the ring labeled A (see Table II and the discussion below). In addition to the amide linkages, a singlet at 5.45 ppm arises from the α-CH of the amino-terminal residue (G-1′).



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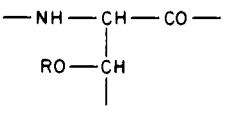

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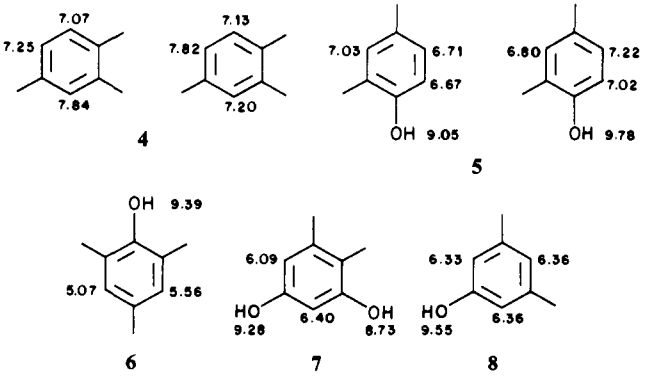
There are seven groups of aromatic protons in the spectrum of **1**, making teicoplanin more similar to ristocetin and related glycopeptides (seven rings) than it is to vancomycin (five aromatic rings). The spectrum of **1**, like the spectra of the ψ-aglycons of A35512B[10] and acetaplanin,[11] contains six phenolic resonances (9.78, 9.55, 9.39, 9.28, 9.06, and 8.73 ppm; 60 °C in Me_2SO-d_6). All six of the phenolic resonances have been assigned by observation of negative NOEs produced at adjacent ring protons when the phenolic peak is irradiated. The phenol at 9.39 ppm is the only one of the six for which no NOE is observed, indicating that this peak arises from a site that has no adjacent protons; two phenols (9.55 and 9.28 ppm) give rise to NOEs at two adjacent sites. The seven groups of aromatic protons and the six phenols are related as in **4**–**8**.



4 **5** **6** **7** **8**

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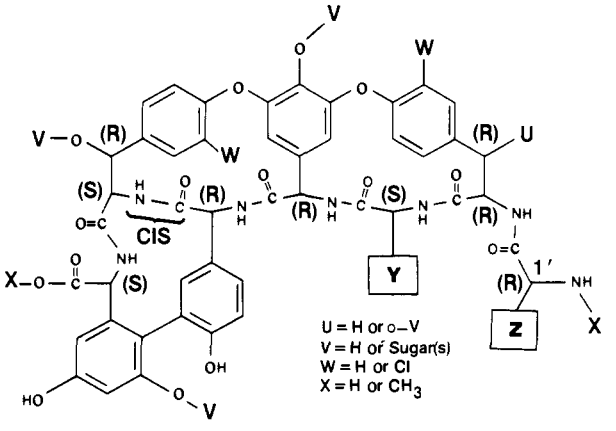
Table I. Proton NMR Assignments[a] for the Teicoplanin Aglycon Methyl Ester, Compared with Corresponding Assignments for A41030B,[b] Actaplanin ψ-Aglycon,[11] A35512B ψ-Aglycon,[10] and Vancomycin[16]

	chemical shifts, δ (J, Hz)				
proton	teicoplanin ψ	A41030B	actaplanin ψ	A35512B ψ	vancomycin
A-NH	6.02 (12)	6.73 (12)	7.49 (11)	7.10 (11)	6.50 (12)
A-2′	4.11 (12)	4.11 (12)	4.26 (~10)	4.31 (11)	4.22 (12)
A-1′	5.27 (~0)	5.08 (~0)	5.07 (~0)	5.10 (~0)	5.13 (~0)
A-2	7.84	7.79	7.70	7.50 (8)	7.87
A-3	Cl	Cl	Cl	6.89 (8)	Cl
A-5	7.07 (8)	7.24 (8)	7.31 (8)	7.15	7.28 (8)
A-6	7.25 (8)	7.43 (8)	7.33 (8,1)	7.41 (8)	7.48 (8)
B-NH	7.47 (8)	7.65 (8)	7.60 (~8)	7.07 (8)	8.14 (8)
B-1′	5.57	5.67 (8)	5.60	5.56 (8)	5.71 (8)
B-2	5.56	5.50	5.63	5.84	5.63
B-4(OH)	9.39		9.47	9.43	
B-6	5.07	5.10	5.03	5.18	5.21
C-NH	8.12 (8)	7.51 (9)	7.98 (8)	7.74 (8)	8.00 (9)
C-2′	4.94	4.97	4.92	5.10	4.86 (4)
C-1′	3.33, 2.88 (14)	3.31 (14,3), 2.84 (14)	3.33, 2.85 (13)	5.12, OH	5.15 (4), OH
C-2	7.82	7.69 (8)	7.85 (8)	7.96 (8)	7.57 (8)
C-3	7.13 (8)	7.25 (8)	6.90 (8,2)	7.07	7.20 (8)
C-5	Cl	Cl	7.19 (8)	~7.14	Cl
C-6	7.20	7.21	7.05 (8)	~7.10	7.42
D-NH	8.47 (5)	8.39 (~5)	9.05	9.06	8.39 (7)
D-1′	4.56 (5)	4.39 (5.5)	4.41 (5.5)	4.39 (5.5)	4.50 (7)
D-2	6.09	6.26 (2)	6.06	6.06	6.30 (2)
D-3(OH)	9.28		9.51	9.56	
D-4	6.40	6.38	6.42 (2)	6.44	6.44 (2)
D-5(OH)	8.73		8.97	8.83	
$COOCH_3$	3.71	COOH	3.73	3.70	COOH
E-NH	8.35 (5)	8.46	8.57	8.77	8.43 (6)
E-1′	4.28 (5)	4.33 (5)	4.49 (6)	4.59 (5)	4.50 (6)
E-2	7.03	7.10	7.19 (1)	7.26	7.19
E-4(OH)	9.06		9.31	9.35	
E-5	6.67 (8)	6.64	6.69 (8)	6.72 (8)	6.73 (8)
E-6	6.71 (8,1)	6.68	6.74 (8, 1)	6.74 (8)	6.78 (8, 1)
F-NH	7.63 (10)	7.73 (10)	7.62 (10)	7.60 (10.5)	
F-1′	5.35 (10)	5.33 (10)	5.27 (10)	5.94 (10.5)	
F-2	6.36	6.38	6.40	6.54	
F-4	6.36	6.32	CH_3	6.66	
F-5(OH)	9.55		9.57	10.59	
F-6	6.33	6.33	6.38	Cl	
G-1′	5.45	4.66	5.51	5.50	
G-2	6.80	6.66	6.72 (1)	6.63	
G-4(OH)	9.78		9.98	10.17	
G-5	7.02	6.93 (8)	7.09 (8)	7.14	
G-6	7.22	7.12	7.19 (8)	7.26	

[a] 360-MHz spectra recorded at 60 °C in Me_2SO-d_6. Chemical shifts are listed vs. internal Me_4Si. Ambient temperature (~23 °C) for A41030B, actaplanin ψ, and A35512B ψ; 70 °C for vancomycin. [b] Data from ref 13 and unpublished results.

Linkage of Substructures To Form 1. The general structure **9** is shared by all the glycopeptides for which structures have been



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published; the groups Y and Z are variable. The glycopeptides inhibit cell wall biosynthesis by binding to mucopeptides containing the terminal dipeptide D-alanyl-D-alanine,[4] and complex formation from the mucopeptide analogue Ac-D-Ala-D-Ala has been demonstrated for many of the glycopeptide antibiotics,[17,18] including teicoplanin.[6] The binding to a common substrate suggests binding sites with similar or identical structural features, and Williams and co-workers have shown that the binding site regions of ristocetin[18a,b] and vancomycin[18b,c] are contained in the common structure **9**. Many of the protons in **9** are held in close proximity, leading to extensive "nests" of NOEs which are repeated in the NMR spectra of each glycopeptide and which are therefore very useful for structure elucidation. Some of these NOE observations are listed in Table II, where data for the teicoplanin ψ-aglycon methyl ester are compared with similar results for actaplanin and vancomycin.

The similarities of both chemical shift and coupling constant data (Table I) and NOE results (Table II) between teicoplanin and the other antibiotics indicate that the substructures **2–8**, the four (NH–CH) pairs, and the peptide terminal groups are linked to form the ψ-aglycon methyl ester **1**. Comparison of the various

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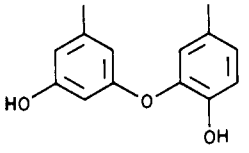
Table II. Selected NOE Observations[a] for 1, Actaplanin ψ-Aglycon,[11] and Vancomycin[22]

proton irradiated	resonances reduced, %		
	1	actaplanin	vancomycin
A-1′	A-2 (+)	A-2 (40)	A-2 (49)
A-2′	A-1′ (+)	A-1′ (27)	A-1′ (32)
A-2′	A-2 (21)	A-2 (37)	A-2 (40)
A-2′	D-NH (+)	D-NH (31)	D-NH (43)
A-2′	E-1′ (43)	E-1′ (41)	E-1′ (35)
A-2′	E-2 (+)	E-2 (27)	E-2 (48)
C-1′[b]	C-2 (20)	C-2 (30)	
E-NH	B-1′ (+)	B-1′ (43)	B-1′ (53)
F-1′	F-6 (12)	F-6 (28)	
F-2	G-2 (18)	G-2 (24)	
G-1′	G-6 (+)	G-6 (28)	

[a] Spectra recorded in Me_2SO at 60 °C for 1, ~23 °C for actaplanin ψ-aglycon, and 35 °C for vancomycin. NOE's that were observed in difference NOE spectra were not quantitated; their observation is indicated by (+). [b] C-1′ proton at 2.88 ppm.

substructures with 1 and 9 shows the following: (a) the two aromatic rings of A are rings A and C, and W = H in 9. (The assumption that W = Cl is confirmed by mass spectrometry and will be discussed below.) The two rings are readily distinguished by the NOE patterns involving proton A-2 (7.84 ppm), including the NOEs involving A-1′ and A-2′ on structure 3; see Table II. Structure 2 is linked to ring C by the NOE C-1′ → C-2 (2.88 → 7.82 ppm) mentioned earlier; structure 2 requires that U = H in 9. (b) The two aromatic rings of 5 are rings E and G in 1, and both NOE observations and chemical shift comparisons allow the two rings to be distinguished. Ring G corresponds to Z in 9; its proximity to the amino-terminal CH (G-1′) is indicated by an NOE, G-1′ → G-6. (c) Structures 6 and 7 have unique substitution patterns when the phenolic assignments are considered; they obviously correspond to rings B and D. (d) Structure 8 must correspond to Y in 9 by process of elimination (ring F in 1).

The teicoplanin NMR results do not provide a direct indication of whether the groups Y and Z are connected to form a diphenyl either (as in ristocetin,[12] actaplanin,[11] etc.) or whether they contain other substituents but are not linked to each other (as in avoparcin,[19] for example). However, structure 8 is identical with the ring F substitution pattern in the A41030 antibiotics,[13] a group of glycopeptides and glycopeptide aglycons; in the A41030 series the groups Y and Z are connected to form the structure 10. If,



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in 9, U = V = X = H, W = Cl, and Y and Z = 10, the resulting structure is that of antibiotic A41030B, $C_{58}H_{45}N_7O_{18}Cl_2$ (integral molecular weight = 1197). If, in the teicoplanin ψ-aglycon methyl ester, U = H, V = H except for *N*-acetylglucosamine, W = Cl, X = H except for the methyl ester, and Y and Z = 10, then the teicoplanin peptide core is the same as A41030B, and the ψ-aglycon ester 1 should have the composition $C_{67}H_{60}N_8O_{23}Cl_2$ (integral molecular weight = 1414). The FAB mass spectrum of 1 supports this identification; $(M + H)^+$ was observed at *m/z* 1415. The true ψ-aglycon of the teicoplanin glycopeptides, therefore, is a peptide identical with A41030B, with *N*-acetylglucosamine attached to the aglycon as the β anomer and linked through the benzylic hydroxyl group at the A-1′ position. Proton NMR parameters for A41030B have been included in Table I for comparison with data from 1.

The absolute configurations shown in 9 were determined by X-ray crystallography for a vancomycin degradation product, CDP-I;[20] differences between vancomycin and CDP-I have recently been described by Harris and Harris.[21] No asymmetric centers are involved in the rearrangement converting vancomycin to CDP-I, and the absolute configurations deduced for CDP-I should be unchanged for vancomycin. The characteristic ability of the glycopeptides to form complexes with the chiral substrate Ac-D-Ala-D-Ala suggests that these molecules, including teicoplanin, share the stereochemical details indicated in 9 for vancomycin. The cis peptide bond in 9 occurs in the CDP-I crystal structure, and NMR results have shown it to be required in all glycopeptides for which the structures have been reported; in vancomycin, for example, if the dihedral angle between the A-2′ and A-NH protons is ~180° ($J_{A\text{-}2'\text{-}NH}$ = 12 Hz)[16] and if the A-2′ and E-1′ protons are in close proximity (NOE ~56%, E-1′ → A-2′),[22] then the amide bond connecting A-2′ and E-1′ must be cis. The teicoplanin data in Tables I and II indicate the presence of such a linkage in 1 as well ($J_{A\text{-}2'\text{-}NH}$ = 12 Hz; NOE ~43%, A-2′ → E-1′).

Groups Attached to 1. The single ψ-aglycon ester 1 was prepared from the complex of teicoplanin glycopeptides (teichomycin A_2 complex), indicating that the teicoplanins differ from each other only in the location or identity of one or more phenolicly linked substitutents. Amino acid analysis of the filtrate obtained after isolation of 1 (see Experimental Section) indicates that one of the components that is removed during formation of the ψ-aglycon is a second glucosamine, and this glucosamine (like the sugar of 1) has a blocked amino group. The NMR spectrum of the teicoplanin complex contains several aliphatic proton resonances between 0.8 and 2.1 ppm; decoupling studies (see below) indicate that the resonances arise from an acyl chain. Linkage of the acyl group to the teicoplanin core occurs through the amino group of the second glucosamine, leading to the amino acid analysis results described above. The presence of acylated glucosamine is also supported by the mass spectral results presented below.

The teicoplanin glycopeptides contain D-mannose in addition to D-glucosamine;[2] mannose is a component in many of the other glycopeptide antibiotics as well.[4] Mass spectra of the teicoplanin complex were examined in the FAB mode, and the results were compared with the expected "non-acylated" molecular weight, 1723 (A41030B + *N*-acetylglucosamine + one mannose + one glucosamine − three waters = 1723). For the complex, prominent peaks occurred at *m/z* 1878 (1877 + H), 1900 (1877 + Na), and 1914 (1891 + Na), and a smaller peak was observed at *m/z* 1892 (1891 + H). After cationization with K^+ there were two prominent peaks at *m/z* 1916 (1877 + K) and 1930 (1891 + K). (On the basis of relative peak heights it appeared that the MW 1877 material was twice as abundant as the MW 1891 material.) These results indicate the presence of acyl chains with the compositions $C_{10}H_{19}O$ for the MW 1877 peak and $C_{11}H_{21}O$ for the MW 1891 component. (The elemental composition computed from the structures of A41030B plus the groups appended to it to form the major teicoplanin factor is $C_{88}H_{97}N_9O_{33}Cl_2$, MW 1877). An additional prominent peak was observed at *m/z* 1563, indicating the loss of acylglucosamine due to fragmentation in the mass spectrometer. This result is similar to observations for vancomycin and derivatives, where the vancomycin disaccharide is readily cleaved from the B-4 phenolic position, producing a spectrum containing peaks for both the parent glycopeptide and the aglycon (J. L. Occolowitz, unpublished results). Loss of the acylglucosamine in this manner, without loss of the mannose, indicates that the two sugars are attached to the teicoplanin aglycon at separate sites, and it suggests that the amino sugar may be attached at the B-4 phenol. This suggestion was supported by examining the NMR spectra of the teicoplanin complex.

The resonances from protons ortho or para to a phenol in a glycopeptide shift downfield by characteristic amounts (~0.3 ppm

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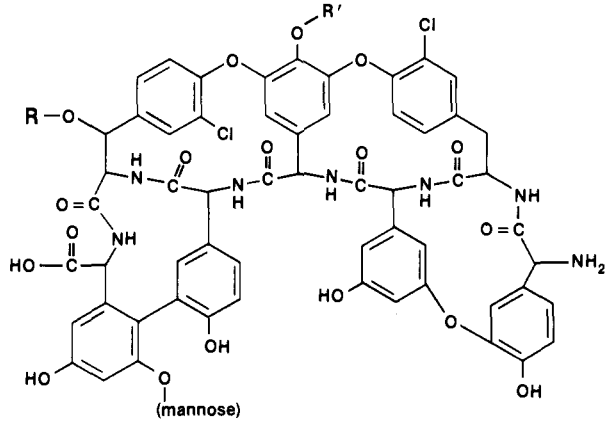
for ortho, ~0.2 ppm for para) when a sugar or an acyl group is linked to the peptide core through the particular phenolic group.[8,10] When the NMR spectra of **1** and the teicoplanin complex are compared, the aromatic resonances for rings E, F, and G show little change in position, indicating that phenols on those rings are not sugar attachment sites in teicoplanin. The ring D resonances, however, are both shifted downfield substantially; D-2 occurs at 6.52 ppm and D-4 at 6.71 ppm in the intact teicoplanin. Part of the chemical shift change for the D-2 proton can be attributed to the influence of the adjacent peptide carboxy terminal, which is $COOCH_3$ in **1** but COOH in the parent teicoplanin. The influence of such a change can be evaluated from Table I. Three of the compounds listed in Table I have methyl esters attached to D-1′: **1**, actaplanin ψ-aglycon, and A35512B ψ-aglycon. The average chemical shifts for the ring protons for the three materials are 6.07 ppm for D-2 and 6.42 ppm for D-4. Two antibiotics in Table I have free carboxyl groups attached to D-1′, A41030B and vancomycin; the average ring D chemical shifts for these two are 6.28 ppm for D-2 and 6.41 ppm for D-4. Comparison of the two sets of averages indicates that hydrolysis of a carboxy-terminal ester attached to D-1′ should cause a downfield shift of ~0.2 ppm for the D-2 resonance but have little or no effect at position D-4. The observed changes on going from **1** to intact teicoplanin are shifts downfield of 0.43 ppm (D-2) and 0.31 ppm (D-4), and these large differences indicate the presence of a sugar on ring D in teicoplanin. Such a linkage is confirmed by the observation of mutual NOEs between the D-4 proton (6.71 ppm) and an anomeric singlet (mannose) at 5.24 ppm; no NOE is observed between 5.24 ppm and proton D-2 (6.52 ppm). These results suggest that the mannose is linked through the teicoplanin D-5 phenol, ortho to proton D-4 (shift ~0.3 ppm for sugar linkage) and para to proton D-2 (shift ~0.2 ppm for sugar linkage plus ~0.2 ppm for absence of the methyl ester); the D-5 phenol is a mannose attachment site for many other glycopeptides as well (ristocetin,[9] actaplanin,[8] and probably A35512B[10]). The ring B phenol has no ortho or para protons, but this is the only site remaining for attachment of the *N*-acetylglucosamine to the peptide core. The teicoplanin factors have differing acyl chains, and they are reported to be converted to a common degradation product at low pH (loss of ~300 units in molecular weight).[6] Sequential hydrolysis studies on the actaplanin glycopeptides have shown that the first major hydrolysis step at low pH is loss of the sugar moiety attached to the B-4 phenol of the aglycon.[8] If the hydrolysis path is the same for the teicoplanins, they should each lose acylglucosamine from position B-4 and be converted to a common product, as is reported (loss of 315 mass units would be expected for the major teicoplanin factor).

The attachment of mannose to phenol D-5 and of acylglucosamine to phenol B-4 of the teicoplanin aglycon leads to **11**



11

as the common structure of the teicoplanin glycopeptides. The resonances of the acyl-chain protons are overlapped to a considerable extent, but decoupling experiments suggest that the $C_{10}H_{19}O$ group on the major teicoplanin factor is an isodecanoyl chain, **12**. The chemical shifts (in ppm) are listed below on the structure.

$$-CO-\underset{2.02}{CH_2}-\underset{1.43}{CH_2}-\underset{1.14\text{–}1.28}{(CH_2)_4}-\underset{1.44}{CH}-\underset{0.83}{(CH_3)_2}$$

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Conclusions

The teicoplanins are new members of the glycopeptide class of antibiotics; they have the general structure **11**. They are unique among the glycopeptides in containing, in addition to sugars and a complex peptide core, a long acyl chain. The factors differ only in the identity of their acyl groups; for the major factor the group is a branched $-C_{10}H_{19}O$ (isodecanoyl). The common teicoplanin aglycon is identical with antibiotic A41030B; attached to this core are three substituents: *N*-acetylglucosamine at the A-1′ benzylic hydroxyl group, *N*-acylglucosamine at the B-4 phenol, and mannose at the D-5 phenol.

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Registry No. **1**, 91032-25-6; **11** (acyl = $-CO(CH_2)_6CH(CH_3)_2$), 91032-26-7; teicoplanin complex, 61036-64-4.

Structure Elucidation of the Teicoplanin Antibiotics

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Abstract: By a combination of chemical, ^1H and ^{13}C NMR, and mass spectrometric studies, the structures of the five major components of the antibiotic teicoplanin, produced by *Actinoplanes teichomyceticus*, have been elucidated. The components all have structures closely related to those of the glycopeptide antibiotics of the vancomycin group. The teicoplanin components are differentiated from one another by the presence of various *N*-acylglucosamine moieties, where the acyl group is a C_{10} or C_{11} fatty acid. Such groups are not present in any other known member of the vancomycin group.

The teichomycins are antibiotics produced by a recently discovered species of actinomycete, *Actinoplanes teichomyceticus*.[1] They have been separated into two major components, teichomycin A_1 and teicoplanin (the major component of which was formerly

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