STRUCTURE AND CONFORMATION OF EPIMERS DERIVED FROM THE ANTIBIOTIC TEICOPLANIN

J. C. J. BARNA, D. H. WILLIAMS, P. STRAZZOLINI,† A. MALABARBA†
and T.-W. C. LEUNG

University Chemical Laboratory,
Lensfield Road, Cambridge, CB2 1EW, U.K.
†Research Laboratories, Gruppo Lepetit S.p.A.,
Via Durando 38, 20158 Milano, Italy

(Received for publication May 8, 1984)

Basic hydrolyses carried out on the glycopeptide antibiotic teicoplanin or its acidic hydrolysis products give rise to epimeric species which retain little antibiotic activity. The detailed structure of a sample epimer has been determined using ^{1}H NMR spectroscopy.

Teicoplanin is a recently discovered[1] complex of antibiotics which has been shown to consist of closely related glycopeptide antibiotics, all belonging to the vancomycin group.[2] Each factor in the complex contains the same aglycone and the same sugar residues, but is differentiated from the other factors by the exact nature of a fatty acid moiety attached to one of the sugars. The structure of the antibiotics is given in Fig. 1.

In the course of structural investigations of teicoplanin (T-A2), acid and base hydrolysis products were prepared; the acid hydrolysis products have been described elsewhere[2,3] and the base hydrolysis (isomerization) products are the subjects of this paper. A representation of the relationship between the species involved is given in Fig. 2. All the transformations with base are simple epimerizations except for the direct production of *epi*-T-A3-1 from teicoplanin. The epimerization of teicoplanin to *epi*-teicoplanin has been carried out on the whole complex and on certain isolated factors. The preparations are described in the Experimental.

All structural work was carried out on the epimer of the pseudoaglycone, *epi*-T-A3-2. It is reasonable to assume that the epimerization found for T-A3-2 occurs analogously for all the other species, whose epimers have been prepared subsequently.

Preliminary evidence that the base hydrolysis products, *epi*-T-A3-1, *epi*-T-A3-2 and *epi*-T-aglycone, were epimers of the acid hydrolysis products was obtained from molecular weight determinations. T-A3-2 was known to be formed from teicoplanin with loss of *N*-acylglucosamines and mannose[3] and was subsequently shown to have the structure given in Fig. 1.[2]

Fig. 1. The structure of teicoplanin (T-A2) and the acid hydrolysis products T-A3-2 and T-aglycone.

T-Aglycone: $R=R_1=R_2=H$

T-A3-2: $R=$ *N*-acetyl-β-D-glucosamine, $R_1=R_2=H$

T-A2 factors (1~5): $R=$ *N*-acetyl-β-D-glucosamine, $R_1=$ *N*-acyl-β-glucosamine (acyl$=C_{10}$ or C_{11} fatty acid), $R_2=$ α-D-mannose

Fig. 2. Schematic representation of the chemical transformations linking teicoplanin with its hydrolysis products.

Teicoplanin (T-A2-1 ~ T-A2-5) —Base 2→ *epi*-Teicoplanin (*epi*-T-A2-1 ~ *epi*-T-A2-5)

Teicoplanin (T-A2-1 ~ T-A2-5) —Base 1→ *epi*-T-A3-1

Teicoplanin —H^+ / −*N*-Acylglucosamines→ T-A3-1

T-A3-1 —Base 1 or 2→ *epi*-T-A3-1

T-A3-1 —H^+ / −Mannose→ T-A3-2; *epi*-T-A3-1 —H^+→ *epi*-T-A3-2

T-A3-2 —Base 1 or 2→ *epi*-T-A3-2

T-A3-2 —H^+ / −*N*-Acetylglucosamine→ T-aglycone; *epi*-T-A3-2 —H^+→ *epi*-T-aglycone

T-aglycone —Base 2→ *epi*-T-aglycone

Base 1 = 3 % aq $NaHCO_3$
Base 2 = 20 % Methanolic 1-butylamine

T-A3-1 was similarly shown to have a structure in which the mannose was still present.[2] The molecular weights of the epimers, determined by fast atom bombardment (FAB) mass spectrometry, were found to be identical with those of the acid hydrolysis products (*i.e.* T-A3-1: 1,562, T-A3-2: 1,400, T-aglycone: 1,197).

Additionally, the ^{1}H NMR spectrum of *epi*-T-A3-2 showed all the resonances found for T-A3-2 but some were significantly shifted relative to those observed for T-A3-2. That this was not merely an accidental result of the pH at which *epi*-T-A3-2 was precipitated in its preparation was shown by adding varying amounts of sodium methoxide to the sample and re-recording the ^{1}H NMR spectrum. The resonances found at significantly different shifts were all due to protons in the right-hand half of the molecule (as shown in Fig. 1). The chemical shifts of all protons in T-A3-2 have been given elsewhere[2] and the absolute values are not important here. Any proton that suffered a relative chemical shift of $\pm$0.15 ppm or more on conversion of T-A3-2 to its epimer is shown with the shift (in ppm) adjacent in Fig. 3, where no stereochemical detail is given. Positive values indicate downfield shifts. (Under nonequivalent pH conditions signals due to other amide protons were also shifted as were those of protons *ortho* to phenolic groups on rings 3 and 7 in Fig. 1). The spectra of *epi*-T-A3-1 and *epi*-T-aglycone showed similar features, but were not analysed in detail.

Fig. 3. Protons in T-A3-2 which show large relative chemical shifts (in ppm) on conversion of T-A3-2 to *epi*-T-A3-2.

The epimerization was shown to occur at the chiral center bearing the proton encircled in Fig. 3,

Fig. 4. Stereochemistry found for *epi*-T-A3-2.

since this proton gave no ^{1}H NMR signal in a sample of *epi*-T-A3-2 prepared using a deuterated medium (see Experimental). The conformation of the epimer was found using ^{1}H-^{1}H nuclear Overhauser effects (NOEs), coupling constants ($^{3}J_{HH}$) and the temperature dependences of the amide protons' chemical shifts. A portion of *epi*-T-A3-2, showing postulated stereochemistry is depicted in Fig. 4.

The most important feature of the epimerization is that it leaves the proton encircled in Figs. 3 and 4 at the front rather than back of the molecule (compare Fig. 1). Hence (in an undeuterated sample) this proton gave an NOE to the adjacent amide proton (in Fig. 4) that was observed to increase rapidly in a study of the time-dependence of the NOEs in this molecule. In the epimerization, the amide group indicated by brackets in Fig. 4 has undergone a rotation through 180°. This was shown by the NOE depicted by in Fig. 4, which was also quickly growing.

Similarly, using the time-dependence of NOEs, it was found that the epimerization had brought about other subtle changes in interproton distances (as indicated by the parameter $t_{1/2}$ for the exponential NOE growth) in the right-hand half of the molecule.

Thus it appeared, for example, that the methylene group adjacent to ring 2 (Fig. 1) had rotated with respect to this ring. Moreover the plane of ring 1 was somewhat nearer to being perpendicular to that of ring 3 than it is in T-A3-2. We have not attempted to derive exact distances for reasons described elsewhere,[2] but all results were consistent with the proposed structure.

Study of the α-CH/NH couplings, $^{3}J_{HH}$, revealed that none had changed by more than 0.6 Hz relative to those for T-A3-2, except the coupling at the epimerized center. Here the value of $^{3}J_{HH}$ changed from ~10.4 to ~7.0 Hz. This piece of data would not have been at all informative, however, in the absence of the evidence from NOEs and deuteration, referred to above.

Finally, the values of the temperature dependences of the NH chemical shifts, $\Delta\delta/\Delta T$, showed quite convincingly that the "cleft" of adjacent amide protons that provides the binding site in the vancomycin group antibiotics[4] has been disrupted, as can also clearly be seen from space-filling molecular models. Values of $\Delta\delta/\Delta T$ for T-A3-2 and *epi*-T-A3-2 are compared in Table 1. The proton nomenclature is that shown in Fig. 1 for T-A3-2. The most significant change is that in the epimer, protons w_2 and w_3 undergo very large changes in chemical shift with temperature. For a sample in dimethyl sulfoxide such values of $\Delta\delta/\Delta T$ indicate that the protons are very exposed to the solvent. In T-A3-2 only proton w_5 was so exposed.

Table 1. Temperature dependences of amide proton chemical shifts in T-A3-2 and its epimer.

Proton	$\Delta\delta/\Delta T$ [a]	
	T-A3-2	*epi*-T-A3-2
w_2	−3.0	−10.0
w_3	−2.5	− 8.7
w_4	−4.5	− 4.0
w_5	−6.0	− 7.5
w_6	−0.8	0
w_7	−2.4	− 2

[a] In ppm/K×10^3; [$^2H_6$]Me_2SO as solvent.

Table 2. *pKa* values of teicoplanins and the related *epi*-compounds in the system MCS - H_2O, 4:1.

Product	pK-1	pK-2
Teicoplanin	5.00	7.10
T-A2-2	4.78	7.03
T-A3-1	4.98	7.12
T-A3-2	4.80	7.10
T-Aglycone	4.78	6.90
epi-Teicoplanin	nd	nd
epi-T-A2-2	4.77	8.27
epi-T-A3-1	4.28	8.49
epi-T-A3-2	4.30	8.43
epi-T-Aglycone	4.12	8.02

nd: Not determined.

Destruction of this binding site was also shown by the lack of effect on the ^1H NMR spectrum of *epi*-T-A3-2 when the bacterial cell wall analogue *N*-acetyl-D-Ala-D-Ala was added.

A concomitant result of the epimerizations is that the *pKa*s of the *N*-terminal amino groups of the teicoplanins are raised significantly (Table 2). A similar change in *pKa* was observed following epimerization of avoparcin[5] but the epimerization demonstrated here for the teicoplanins is not analogous to that proposed for avoparcin[6]. We presume that the epimerizations of the teicoplanins are driven to completion by the greater thermodynamic stability of the products, associated with the more normal, alternating, conformation of the peptide backbone which contrasts with the strained structure needed to form the binding site in the intact antibiotics and acid hydrolysis products.

Experimental

FAB mass spectra were recorded on a Kratos MS-50 instrument fitted with a standard FAB source and a high field magnet. The sample ($\sim$10 nmol) was dispersed in a few μl of α-thioglycerol - diglycerol (1: 1) matrix and bombarded with a 6~9 keV beam of Xe atoms.

^1H NMR spectra were obtained using a modified Bruker WH400 spectrometer operating in the Fourier transform mode. Solutions were $\sim$14 mM in $[^2H_6]Me_2SO$, prepared from samples previously dried *in vacuo* over P_2O_5. Spectra were obtained over the temperature range ambient to $\sim$70°C.

HPLC analyses were performed with a Hewlett Packard model 1084 B machine equipped with a 254 nm wavelength detector and a Zorbax ODS, 5 μm, 250×4 mm prepacked column. Gradients of 0.025 M NaH_2PO_4 - MeCN at pH 6.0 were used.

Potentiometric titrations were performed by dissolving about 20 mg of the compounds in 8 ml of Methyl Cellosolve (MCS) - H_2O, 4: 1 and then adding an excess (2 ml) of 0.01 M HCl to the same solvent mixture. The titrations were made with 0.01 M NaOH in MCS - H_2O, 4: 1.

Basic Hydrolysis of Teicoplanin to *epi*-T-A3-1

T-A2 (6 g) was added in one batch to 570 ml of 3% aq $NaHCO_3$ and 30 ml of 1-BuOH (preheated to 80°C) with vigorous stirring. The pH of the resulting solution was 8.4. The reaction mixture was heated under reflux (bath temp 120°C) for 5 hours (final pH 10.5), cooled to 10~15°C and 2 M HCl was added to give pH 1.2. 1-BuOH (1.2 liters) was added to the mixture and the resulting suspension was concentrated (bath temp 55°C, under vacuum) until the water had evaporated. Et_2O was added and the precipitate was collected and re-dissolved in 300 ml of MeOH. Silica gel (15 g) was added and the solvent removed. The residue was chromatographed on a column containing 150 g of silica gel in MeCN. The column was developed using a gradient of MeCN - H_2O (100% MeCN to 50: 50, MeCN - H_2O). The eluates were monitored by HPLC. Addition of 1-BuOH (2 liters) to the MeCN - H_2O, 50: 50 eluate was followed by concentration to remove MeCN and H_2O. Addition of Me_2CO and Et_2O was followed by collection of a precipitate and washing with Et_2O. Drying of the precipitate gave 0.74 g of *epi*-T-A3-1.

Basic Isomerization of T-A3-1 to *epi*-T-A3-1

T-A3-1 (0.1 g) was added to a mixture of 9 ml of 3% aq $NaHCO_3$ and 1 ml of 1-BuOH. After heating the mixture under reflux for 45 minutes the mixture was cooled and checked by HPLC, at which point some T-A3-1 remained and 0.07 g of *epi*-T-A3-1 had been formed. Further heating under reflux for 70 minutes gave no *epi*-T-A3-1 or *epi*-T-A3-2, only unidentified degradation products.

Basic Isomerization of T-A3-2 to *epi*-T-A3-2

T-A3-2 (2.5 g) was added in one bath to a stirred mixture of 180 ml of 3% aq $NaHCO_3$ and 20 ml of 1-BuOH preheated to 90°C. The reaction mixture was refluxed 45 minutes (bath temp 120°C). After rapid cooling to 10~15°C, 350 ml of a mixture of H_2O - MeCN - 0.2% aq HCO_2NH_4, 45: 45: 10 was added and the pH adjusted to 5.3 with 2 M HCl. The precipitate formed was collected, washed with 50 ml of H_2O and re-dissolved in a solution of 6 ml of 1 M HCl in 300 ml of MeCN. Charcoal (5 g) was added and the resulting suspension was stirred for 30 minutes at room temp, then filtered.

The charcoal was extracted with 20 ml of MeOH. The combined filtrates were concentrated under vacuum. A mixture of H_2O - MeCN, 79: 21 (550 ml) was added and the pH brought to 5.4 with 1 M NaOH. The precipitate was collected, washed with 200 ml of MeOH and then with Et_2O. After drying at 65°C *in vacuo*, 1.59 g of pure *epi*-T-A3-2 was obtained.

Basic Isomerization of T-A3-2 in a Deuterated Medium

The above preparation was repeated using 0.05 g T-A3-2, with D_2O and 1-butan-*d*-ol in appropriate quantities, to give 0.015 g of D-*epi*-T-A3-2.

Acid Hydrolysis of *epi*-T-A3-1 to *epi*-T-A3-2

Epi-T-A3-1 (0.02 g) was added to a stirred solution of 2 ml of 1 M HCl preheated to 80°C. The reaction mixture was heated under reflux at 80°C for 30 minutes, monitoring the reaction by HPLC. After 30 minutes the yield of *epi*-T-A3-2 was found to be 0.012 g.

Epimerizations using Methanolic Butylamine

All teicoplanins were epimerized as described below. In the case of T-A3-1 and T-A3-2, this represents an alternative to the method using aq $NaHCO_3$.

Teicoplanin (complex, factor or acid hydrolysis product) was dissolved in 20% methanolic *n*-butylamine (10 mg/ml) and heated under reflux (bath temp 80°C) for 3~6 hours. Then the reaction mixture was concentrated to dryness and the residue was suspended in MeOH and brought to dryness again. The residue was dissolved in 0.1 M HCl (volume as for reaction mixture) and the pH was brought to 6.5 with 1 M NaOH. The precipitate which formed was filtered, washed with H_2O, a little MeOH then Et_2O, collected and dried. Yields were 80%.

Acknowledgments

We thank the SERC for financial support.

References

1) BORGHI, A.; C. CORONELLI, L. FANIUOLO, G. ALLIEVI, R. PALLANZA & G. G. GALLO: Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. IV. Separation and characterization of the components of teichomycin (teicoplanin). J. Antibiotics 37: 615~620, 1984
2) BARNA, J. C. J.; D. H. WILLIAMS, D. J. M. STONE, T.-W. C. LEUNG & D. M. DODDRELL: Structure elucidation of the teicoplanin antibiotics. J. Am. Chem. Soc., in press
3) MALABARBA, A.; P. STRAZZOLINI, A. DEPAOLI, M. LANDI, M. BERTI & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VI. Chemical degradation: Physico-chemical and biological properties of acid hydrolysis products. J. Antibiotics 37: 988~999, 1984
4) WILLIAMS, D. H.; M. P. WILLIAMSON, D. W. BUTCHER & S. J. HAMMOND: Detailed binding sites of the antibiotics vancomycin and ristocetin A: Determination of intermolecular distances in antibiotic/substrate complexes by use of the time-dependent nOe. J. Am. Chem. Soc. 105: 1332~1339, 1983
5) FESIK, S. W. & I. M. ARMITAGE: NMR studies on the antibiotic avoparcin: Conformational properties in relation to antibacterial activity. Mol. Pharmacol. 25: 275~280, 1984
6) ELLESTAD, G. A.; R. A. LEESE, G. O. MORTON, F. BARBATSCHI, W. E. GORE, W. J. MCGAHREN & I. M. ARMITAGE: Avoparcin and epiavoparcin. J. Am. Chem. Soc. 103: 6522~6524, 1981