TEICOPLANIN, ANTIBIOTICS FROM *ACTINOPLANES TEICHOMYCETICUS* NOV. SP.

VII. PREPARATION AND NMR CHARACTERISTICS OF THE AGLYCONE OF TEICOPLANIN

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Several hydrolytic reactions that transform teicoplanin or its pseudo-aglycones into the aglycone with good yields are described. The most interesting approach is hydrolytic removal of the sugars in benzyl alcohol with the formation of the aglycone benzyl ester which is then submitted to hydrogenolysis. A detailed description of the ^{1}H and ^{13}C NMR spectra of the teicoplanin aglycone hydrochloride is presented. All the signals were attributed to the hydrogen and carbon atoms using homo and heteronuclear COSY. The relevant interactions through space between the hydrogen atoms were obtained by NOE. The structural aspects are discussed in terms of the well-known mechanism of action of the glycopeptide antibiotics.

Teicoplanin (**I**), a new glycopeptide antibiotic belonging to the vancomycin and ristocetin group[1,2], is produced by fermenting a strain of *Actinoplanes teichomyceticus* ATCC 31121[3]. It is formed by a complex of five closely related factors (T-A2-1 ~ 5) and one, more polar factor designated T-A3-1 (Fig. 1)[4].

Structural investigations[5~7] showed that each factor contains the same core-peptide carrying a D-mannose, an *N*-acetyl-D-glucosamine and an *N*-acyl-glucosamine. The different fatty acids constituting the acyl moieties of the latter differentiate the factors.

Hydrolysis studies[5] revealed that factor T-A3-1 is a pseudo-aglycone which is obtained from the complex by selective hydrolysis that removes the *N*-acyl-glucosamines. Further hydrolytic treatment leads to a second pseudo-aglycone (T-A3-2) that lacks the D-mannose and, finally, to the teicoplanin-aglycone (T-aglycone, **II**) (Scheme 1).

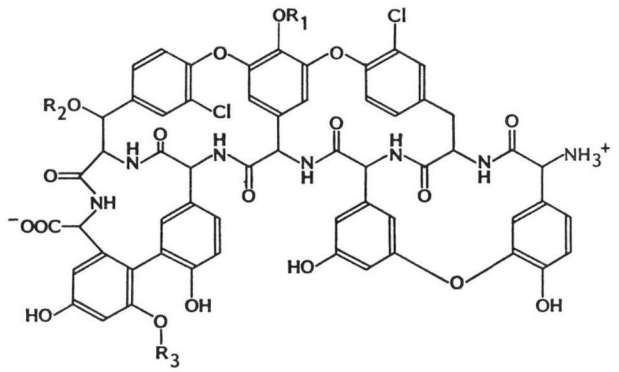
In this report some different methods for the preparation of suitable amounts of **II** are described together with a detailed study of its ^{1}H and ^{13}C NMR spectra.

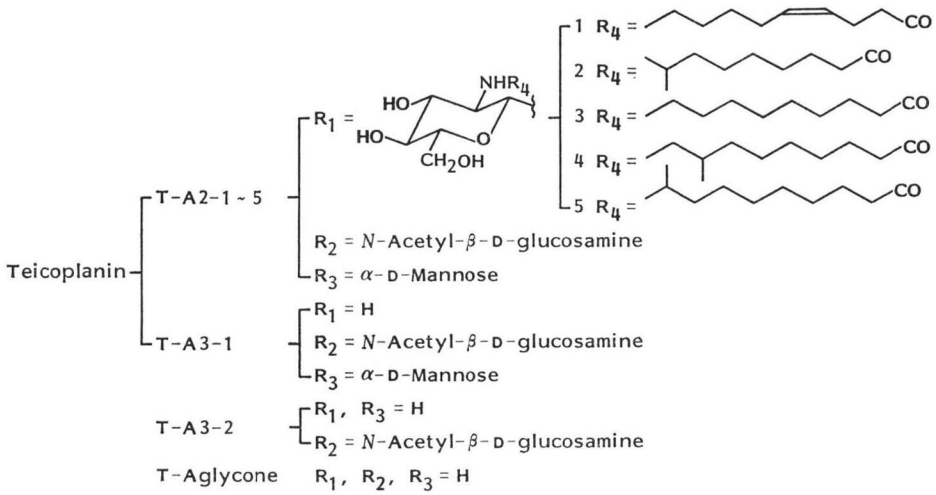
Chemistry

The importance of the pseudo-aglycones and of the aglycones of the vancomycin-type glycopeptides has been recognized in many papers and patent applications. While in the past the preparation of hydrolysis products has been intended mainly for structural studies, at present the interest in their intrinsic antibacterial activity and as substrates for chemical modifications is emerging[5,8~15].

In a few cases only the aglycones were obtained directly from fermentation: *Streptomyces virginiae* NRRL 15156 produce A41030 antibiotic which is a complex containing four different sugar-lacking glycopeptides[16] and *Streptomyces toyocaensis* NRRL 15009 produce antibiotic A47934 which is an aglycone sulfate ester[17]. Recently, agluco-vancomycin and the aglycones of antibiotics A51568A

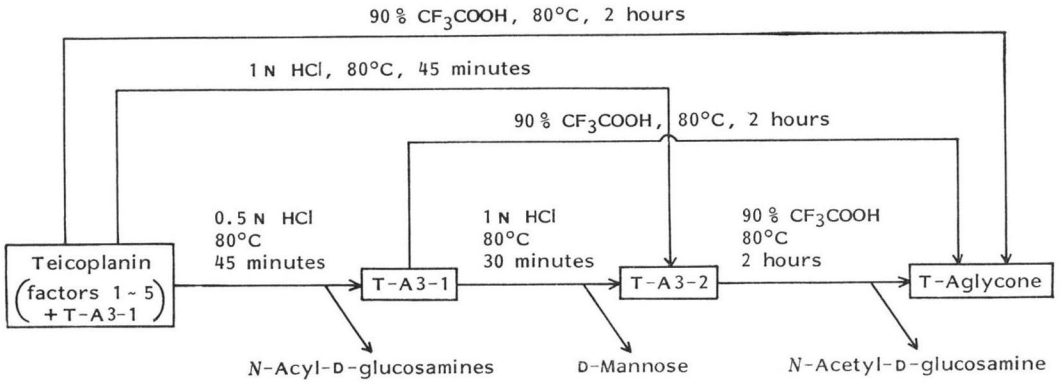
Fig. 1. Structures of the components of teicoplanin, the pseudo-aglycones and the aglycone.





Scheme 1.

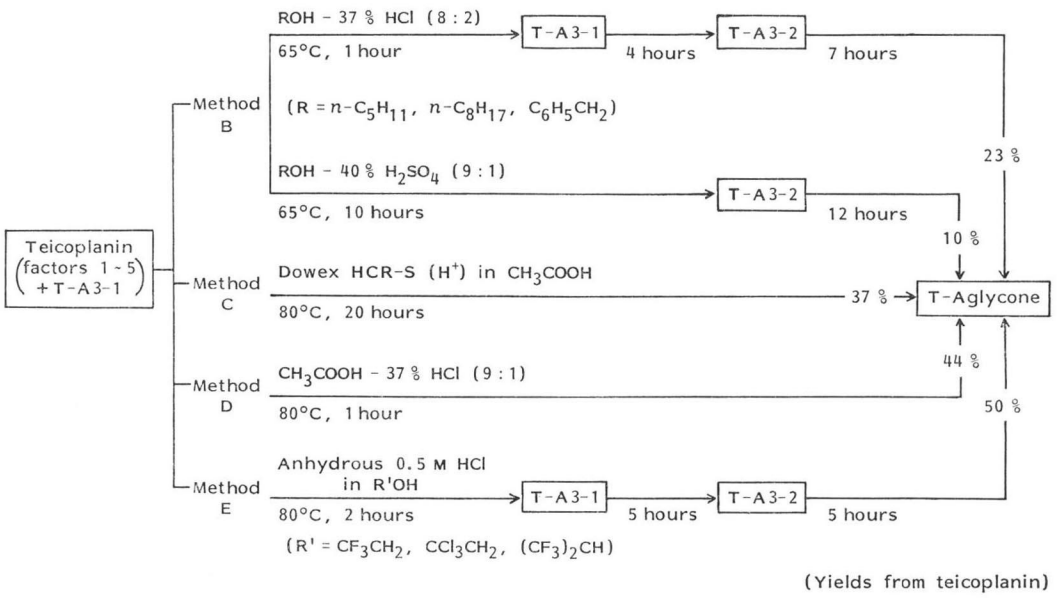
Method A



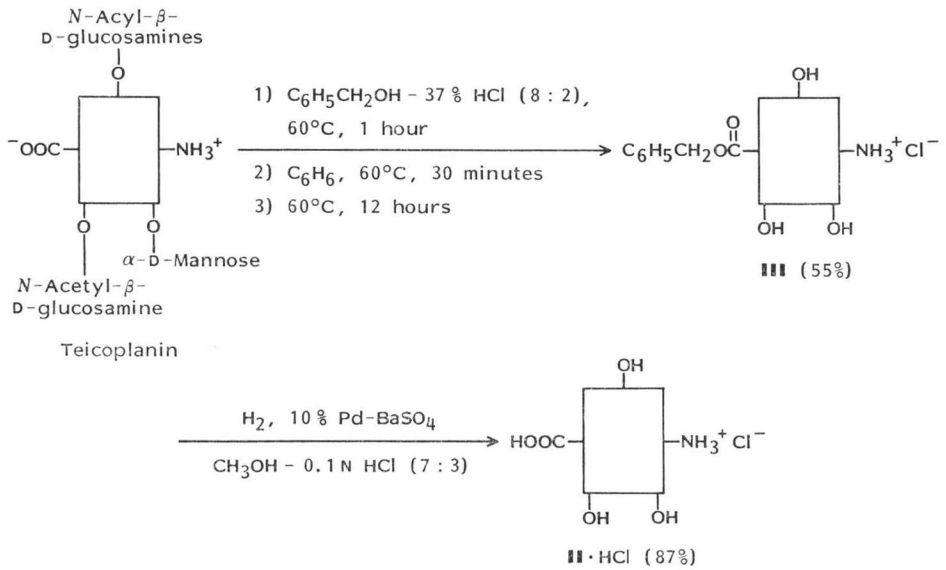
and M43A have been isolated from fermentation broth of *Nocardia orientalis* NRRL 2452[18].

It is evident from the literature above that no general hydrolysis procedures can be established because the removal of sugars depends upon many factors such as the stability and the character of

Scheme 2.



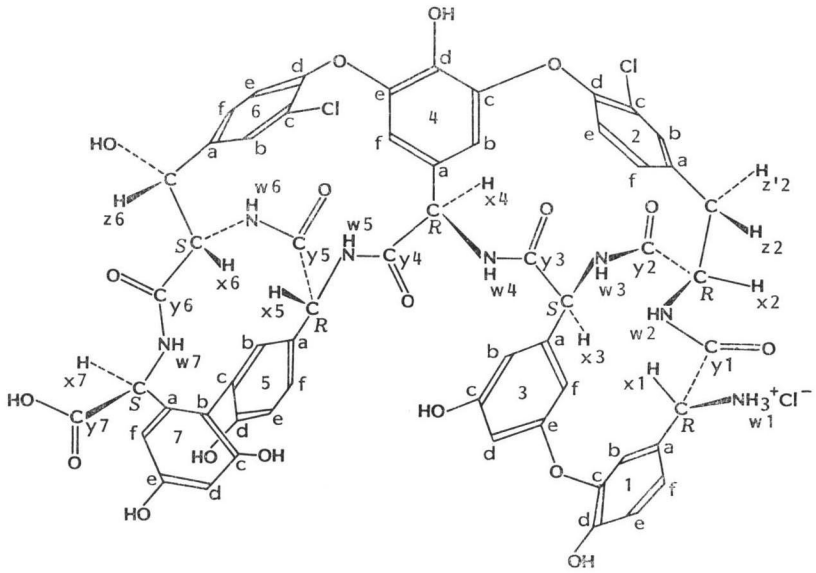
Scheme 3.



the acetalic bonds, the steric hindrance, the type of the sugars and the presence of carboxylic esters. Particular attention has to be paid to avoid epimerization[11,19,20] or unwanted rearrangements[8].

Treatment of teicoplanin (**I**) with 90% aq CF_3COOH gave the aglycone (**II**) in a 16% yield (Method A, Scheme 1). Alternative methods are shown in Scheme 2. **I** was suspended in benzyl alcohol (or other alkanols slightly miscible with water) in the presence of concd HCl. By heating for 12 hours, **II** was obtained in a 23% yield. The yields were lower when using 40% aq H_2SO_4 (Method B). In a different approach, by heating a solution of **I** in acetic acid in the presence of Dowex HCR-S resin higher

Fig. 2. Structure of the teicoplanin-aglycone (**II**) hydrochloride, configuration (*R* or *S*) of the asymmetric centers, and nomenclature proposed by the D. H. WILLIAMS group[7] and used in this paper.



yields were obtained (37%) (Method C). The use of concd HCl instead of the resin gave a 44% yield (Method D). Better results were obtained by treating a suspension of **I** in trifluoroethanol or in other not easily esterificating alcohols in the presence of gaseous HCl (water for the hydrolysis is originally present in teicoplanin as residual solvent). After heating for 12 hours, a 50% yield was obtained (Method E).

When Method A was applied to the partial hydrolysis products T-A3-1 and T-A3-2, **II** was obtained with 45% and 19% yields, respectively. Also Methods B and E may be applied to the intermediate derivatives T-A3-1 and T-A3-2. When starting from **I**, their formation was checked by HPLC analysis. The various hydrolysis methods required relatively different purification procedures.

An approach that gives **II** in a good yield (47%) with high purity is shown in Scheme 3. When a solution of **I** in benzyl alcohol - concd HCl (as in Method B) was heated under vacuum with repeated additions of benzene, the benzyl ester of T-aglycone (**III**) formed; this was isolated and submitted to catalytic hydrogenolysis. **II** was obtained by chromatographic purification in a crystalline form (needles) which unfortunately was not suitable for X-ray analysis. On the other hand, purified **II**·HCl gave very good NMR spectra.

Spectroscopic Studies

The ^{1}H and ^{13}C NMR spectra together with the various NMR experiments were obtained with Bruker instruments at 500 MHz and 100.61 MHz, respectively, both equipped with an Aspect 3000 console.

The spectra were recorded at 40°C in DMSO-d_6 solution (internal standard TMS, $\delta=0.00$ ppm). The samples were previously lyophilized twice from DMSO-d_6 solutions. The spin-spin interactions were observed and/or measured by homonuclear or heteronuclear 2D COSY (correlation spectroscopy). The results of NOE (nuclear Overhauser effect) measurements have only qualitative significance be-

Table 1. Assignments of the signals of the ^1H NMR spectrum of the teicoplanin-aglycone (**II**) hydrochloride (labelling system Fig. 2).

Proton	δ (ppm)	^3J (Hz)	Proton	δ (ppm)	$^{2,3,4}J$ (Hz)
x1	5.47	nd	z2	2.87	14 and 3
x2	4.92	nd	z'2	3.35	14 and 5
x3	5.35	10	z6	5.10	nd
x4	5.60	8	1b	6.77	2
x5	4.33	5	1e	7.02	8
x6	4.10	12 and 2	1f	7.20	8 and 2
x7	4.42	6	2b	7.20	2
w1	8.53	nd	2e	7.16	8
w2	8.10	8	2f	7.86	8 and 2
w3	7.66	10	3b	6.32	2
w4	7.53	8	3d	6.34	2
w5	8.38	5	3f	6.39	2
w6	6.62	12	4b	5.50	2
w7	8.40	6	4f	5.08	2
OH-z6	5.85	6	5b	7.07	2
OH-1	9.88	—	5e	6.65	8
OH-3	9.63	—	5f	6.68	8 and 2
OH-4	9.45	—	6b	7.77	2
OH-5	9.08	—	6e	7.19	8
OH-7c	8.78	—	6f	7.43	8 and 2
OH-7e	9.34	—	7d	6.39	2
			7f	6.24	2

nd: Not determined.

cause they were obtained under non optimized conditions. Long range C-H correlations from COLOC (correlation *via* long range coupling) experiments were obtained with D3=40 msec and 30 msec and the data read from cross sections through each carbon.

The detailed structure of **II**·HCl together with the nomenclature system proposed for this molecule by D. H. WILLIAMS group[7] is reported in Fig. 2 and will be referred to throughout this work for the NMR assignments. Residues are numbered starting at the *N*-terminus: α carbons/protons are designated x_n; β carbons/protons z_n; CO groups y_n; amide protons w_n; lettering of aromatic carbons/protons is such that substituted carbons are reached first in the lettering system and in case of symmetry the lettering is counterclockwise. This nomenclature system should be much welcomed among the researchers in the glycopeptide antibiotics field because it gives an authoritative uniformity based on a simple and rational way of labelling. Unfortunately, this system is somewhat different from the one used in biochemistry where the amino acids are numbered from the one bearing the carboxylate ion. In any case, a unique nomenclature will certainly help a lot in reading

Table 2. Selected qualitative difference NOE observations for teicoplanin-aglycone (**II**) hydrochloride protons (labelling system Fig. 2).

Proton irradiated	Resonance involved
x2	z2, z'2
x3	3b
x4	4b, 4f, w5
x5	x6, 4f, z6, 5b, 6b
x6	x5, z6, 5b, 6b, w7
x7	7f, 5b
z2	x2, 2f, w2
z'2	x2, 2b
5e, 5f	OH-5
6b	z6, x5, x6, w7
w2	w3, 2f, x1, z2, 1b, 3f
w3	w2, w4, 1b, 3f, 4b
w4	w3, 3f, 2b, 4b
w5	x4, 4f, 5f
w7	5b, 6b, 7f, x5, x6

Table 3. Assignments of the signals of the ^{13}C NMR spectrum of the teicoplanin-aglycone (**II**) hydrochloride (labelling system Fig. 2).

Carbon	δ (ppm)	$^1J_{CH}$ (Hz)	Carbon	δ (ppm)	$^1J_{CH}$ (Hz)
x2	36.06	nd	6f	127.42	165
x5	53.56	137	4a	127.91	q
x1	54.47	146	2f	130.57	165
x4	54.69	142	2b	131.04	166
x2	55.57	146	4d	134.25	q
x7	56.55	140	2a	135.26	q
x3	58.21	143	5b	135.68	158
x6	61.73	140	7a	135.96	q
z6	71.49	150	3a	140.73	q
3f	102.60	162	6a	142.14	q
7d	103.71	164	1c	142.69	q
4f	104.45	160	4e	147.43	q
3d	105.02	161	4c	147.86	q
7f	105.80	162	6d	148.70	q
4b	107.69	160	1d	149.24	q
3b	109.90	160	2d	150.90	q
5e	116.59	159	5d	155.45	q
7b	117.77	q	7c	156.46	q
1e	118.27	165	7e	157.21	q
1b	119.98	158	3e	158.01	q
5c	121.16	q	3c	158.48	q
1a	122.63	q	y6	167.62	—
6e	123.14	165	y3	168.26	—
2e	124.78	165	y1	168.54	—
5f	125.79	162	y2	168.88	—
5a	125.92	q	y5	169.19	—
6c	126.26	q	y4	169.95	—
1f	126.68	166	y7	169.43	—
6b and 2c	127.19	166 and q			

nd: Not determined.
q: Quaternary carbon atom.

the scientific papers in this field by avoiding boring and tedious decodifications of the numerous different labelling systems used up to now. The assignments of the proton signals are based on comparison with data for teicoplanin and other glycopeptides[6,7,11,12,21~24], on ^1H-^1H COSY 45 and on one dimension NOE observations. The proton assignments are listed in Table 1 and the NOE observations in Table 2.

The ^{13}C assignments are based on comparison with data for other glycopeptides[25~28], on DEPT (distortionless enhancement by polarization transfer), on ^{13}C-^1H COSY, on ^{13}C-^1H coupling J resolved, and on ^{13}C-^1H COLOC experiments. The carbon assignments are listed in Table 3.

Assignments of the Proton Signals

^1H NMR data have already been reported for A41030B[6], a substance corresponding to **II**. It is worthwhile to say that **II** in DMSO solution is not ionized, *i.e.*, it does not exist as internal salt (zwitterion). Conversely and obviously, **II**·HCl in DMSO solution exists in the protonated form. From the published data of **II** and those obtained on **II**·HCl it was possible to assign all the 46 protons but one of **II**·HCl and to reach the following conclusions:

i) The spectrum of **II**·HCl shows sharper signals than those of **II**.

Table 4. Effect of deprotonation of the amino group on the surrounding protons (chemical shift, $\Delta\delta$, ppm).

	II·HCl	II[6]	$\Delta\delta$
x1	5.47	4.66	0.81
1b	6.77	6.66	0.11
2f	7.86	7.69	0.17
w2	8.10	7.51	0.59

Table 5. Long range couplings (^4J and ^5J) observed by ^{1}H-^{1}H COSY-45.

^5J	^4J
OH-1 - 1b	z2 - 2b and 2f
OH-1 - 1f	x3 - 3b and 3f
OH-4 - 4b	x4 - 4b and 4f
OH-4 - 4f	x5 - 5b and 5f
OH-5 - 5b	z6 - 6b and 6f
	x7 - 7f

ii) The signals of the phenolic OH groups in **II** are not resolved enough to give their individual assignments, while in **II**·HCl they are sharp and well resolved. This fact is interpreted as due to a different rate of proton exchange, namely slower for **II**·HCl than for **II**. The alcoholic OH group is well detected in **II** and in **II**·HCl, while the proton of the carboxylic group is not.

iii) For the same reason of proton exchange rate the NH_2 group does not give rise to a signal in **II** but the NH_3^+ group is well detected in **II**·HCl.

iv) The protons surrounding the amino group undergo upfield shifts on deprotonation of **II**·HCl. In fact, protons x1, 1b, 2f and w2 show shifts of 0.81, 0.11, 0.17 and 0.59 ppm, respectively (Table 4). From these data and on the basis of CPK models of teicoplanin aglycone it should be deduced that as the amino group is close to 2f, the *S* configuration should be assigned to C-x1. In fact, analogous interactions found in ristocetin first led to this conclusion[29], but further structural studies established the *R* configuration for C-x1. The explanation is that the above interactions are possible since one of the two conformations obtained by a 180° rotation of the amide between amino-acids 1 and 2 allows such interactions[30].

v) The assignment of the six phenolic OH's was made primarily for OH-1, OH-4 and OH-5 through the long range ^5J HH observed by COSY (Table 5). These attributions were confirmed by using the findings obtained by the COLOC experiment which assigned the remaining OH groups. With this experiment, in fact, it was possible to assign, among the others, all the quaternary carbons bearing the phenolic OH's. Successively, through the ^2J of the C-O-H groups, which were also detected by COLOC, the OH signals were assigned.

vi) With the COSY experiment seven CH-NH coupling were detected, two of which are part of a 3 spin system HN-CH-CH, *i.e.*, the two phenylalanine moieties 2 and 6. Thus, couplings are clearly observed for w2-x2-z2-z2′ and w6-x6-z6. The sequence of amino acids in the peptidic chain was based on the NOE data (see Table 2) and the long range couplings (^4J) between peptidic (C-H) and aromatic protons, obtained as weak signals from COSY, and observed for all the aromatic rings except ring 1 (Table 5).

vii) The NOE observations (Table 2), which indicate the interactions through space for the teicoplanin aglycone molecule, fit perfectly the stereochemical Dreiding model shown in Fig. 3. This shape of the molecule is currently described[1,2,23,31~33] as responsible for the complex formation between the glycopeptide and *N*-acetyl-D-Ala-D-Ala, the molecule mimicking the binding site of the bacterial cell-wall during the biosynthesis, thus explaining the mechanism of action of these antibiotics at a molecular level.

Assignment of the Carbon Signals

By means of a series of NMR experiments it was possible to assign the signals of all the 58 carbon

Fig. 3. Stereo-structure of teicoplanin-aglycone (II) obtained from a Dreiding model. The interactions through space found by the NOE experiment (Table 2) can be seen.

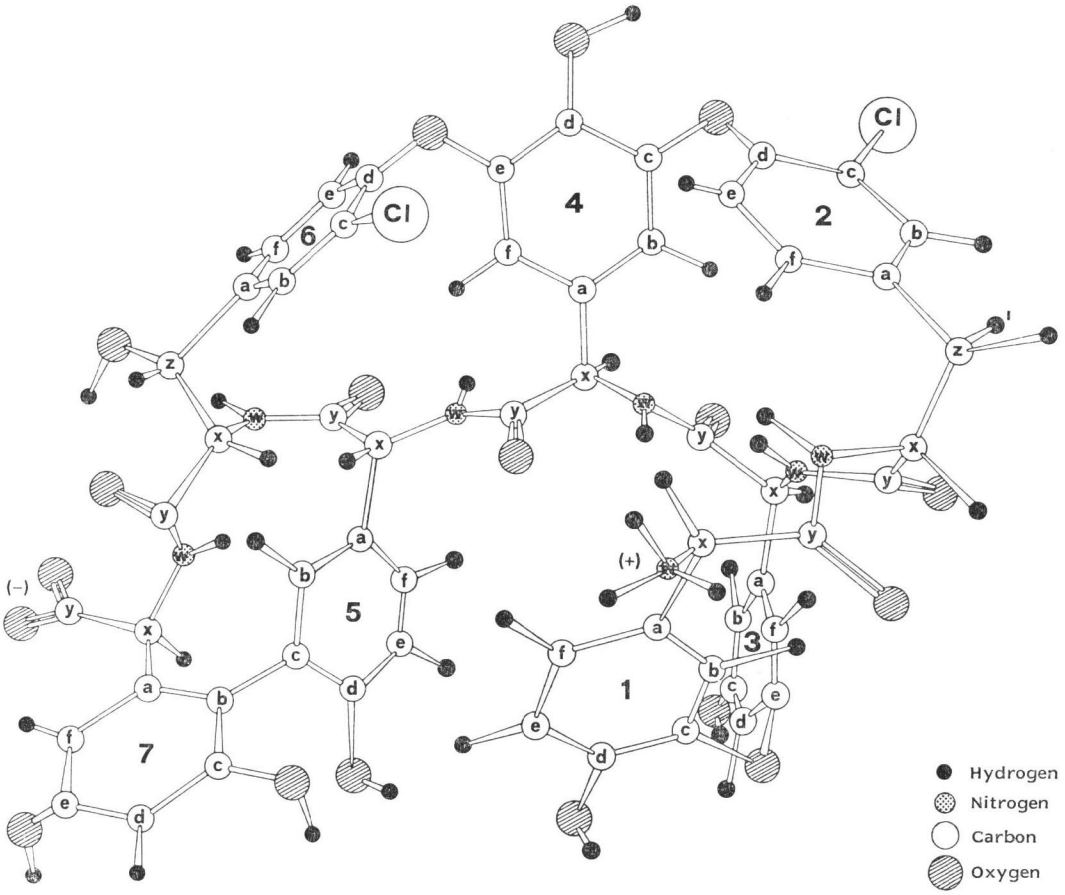


Table 6. Long range coupling (^2J and ^3J) observed by ^{13}C-^1H COLOC (30 and 40 ms) for quaternary and carbonyl carbons.

Carbon	Proton		Carbon	Proton		Carbon	Proton
	^2J	^3J		^2J	^3J		^2J
1a	x1	1e	5a	5f, x5	—	y1	x1
1c	1b	1e, OH-1	5c	—	OH-5	y2	x2
1d	—	1b, 1f	5d	5e	5b	y3	x3
2a	2b	x2	6a	—	6e	y4	x4
2c	—	2e	6c	6b	6e	y5	x5
2d	—	2b, 2f	6d	—	6b, 6f	y6	w7
3a	x3	—	7a	x7	—	y7	x7
3c	3d, OH-3	—	7b	—	7f, OH-7c		
3e	3d, 3f	—	7c	7d, OH-7c	—		
4a	4b, 4f, x4	—	7e	7d, OH-7e	—		
4c	4b	OH-4					
4d	—	4b					
4e	4f	OH-4					

atoms of **II**·HCl. This is being for the first time in the case of the glycopeptides of the vancomycin-ristocetin group. From the DEPT sequence the signal of the only CH_2 group was easily attributed. The fully decoupled ^{13}C NMR spectrum was obtained with well resolved signals, among which it was possible to distinguish quaternary carbons from those bearing protons by DEPT and to determine the J value by using the C-H coupling ^1J resolved experiments. $^1J_{CH}$ values of about 140 Hz for aliphatic CH's and of about 160 Hz for aromatic CH's were obtained. The $^1J_{CH}$ COSY was used to attribute all the carbons bearing protons and this was based on the already known proton attributions.

The last step was the use of the COLOC experiment, which is also dependent from a previous proton assignment; on the basis of the long range $^2J_{CH}$ and $^3J_{CH}$ values all the quaternary aromatic and carbonyl carbons were assigned (Table 6). For the peptide sequence, only the junction between amino acids 6 and 7 was made evident by the present COLOC experiment. In any case, a deeper investigation of this point will be made in the future.

Besides the quaternary carbons, it was possible to assign by COLOC the six phenolic OH's, taking advantage of the long range coupling $^2J_{C-OH}$.

The 2D ^1H NMR spectrum of T-aglycone benzyl ester hydrochloride (**III**) was recorded in DMSO-d_6 solution with a Bruker Instrument equipped with an Aspect 3000 console at 250 MHz. Upon comparison of the data it appears that all the signals fall between ± 0.03 ppm in respect with those of **II**·HCl, except for the signals attributed to x7, 7d and 7f which show shifts of $+0.15$, $+0.07$ and -0.07 ppm, respectively, due to the effect of the benzylic esterification of the terminal carboxyl group. In addition, there are the signals at δ 5.19 (s, 2H) and 7.32 (m, 5H).

Discussion

The biological activity of teicoplanin-aglycone (**II**) has already been discussed[5]. The compound is more active *in vitro* than teicoplanin, particularly against *Staphylococcus epidermidis* strains, and shows also a certain degree of activity against Gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*). *In vivo*, however, it is less active than the parent antibiotic complex.

The mechanism of action of teicoplanin, as for the other members of the class, is the inhibition of the biosynthesis of the bacterial cell-wall by interfering with the mucopeptides terminating with the dipeptide D-Ala-D-Ala at the free carboxyl. A complex is formed that is stabilized by hydrogen bonds between the NH groups and one carbonyl of the glycopeptide and the carboxyl group, one carbonyl and one NH group of the D-Ala-D-Ala terminal. In recent papers[32,33] the free energies of binding for T-aglycone (**II**) and pseudo-aglycones T-A3-1 and T-A3-2 with a model peptide *N*-Ac-D-Ala-D-Ala were measured. It appears that the removal of the carbohydrate moieties do not significantly influence the binding-associated activity. Anyway, data on teicoplanin[5] and on aridicin[15] demonstrated that the fatty acid residues and the sugars strongly influence the *in vivo* activity.

Finally, using the high field NMR spectroscopy and the modern pulse sequences a complete ^1H and ^{13}C NMR spectral assignment of **II**·HCl was made. This constitutes a basis for the assignment of the NMR spectra of other teicoplanins or teicoplanin-like antibiotics of natural or semi-synthetic origin and for discussing their biological activity at a structural level.

In addition, together with the FAB-MS studies published on a large number of glycopeptides[34], it offers a powerful reference for the structural determination and fast recognition of new glycopeptides obtained by screening procedures.

Experimental

Evaporation was carried out with a rotary evaporator at 45°C (bath temperature) under vacuum. Column chromatographies were done on Silanized silica gel 60, 0.06~0.2 mm (Merck).

UV spectrum was recorded on a Unicam SP 800 spectrometer.

HPLC were run with a Varian Model 5000LC pump equipped with a 20 μl loop injector Rheodyne Model 7125 and a Perkin-Elmer LC15 detector at 254 nm. Column; pre-column (5 cm) packed with Perisorb RP-8 (30 μm) Merck followed by a column Hibar RT 250-4 Merck prepacked with LiChrosorb RP-8 (10 μm). Eluent; CH_3CN - 0.2% aq $HCOONH_4$, linear step-gradient ranging from 10% CH_3CN to 30%. Injection; 20 μl. Flow rate; 2 ml/minute. The reactions were monitored by injecting samples of the solutions diluted enough to obtain a final concentration of 1 mg/ml. Final products were checked by injecting solutions of 10 mg each of the hydrolysis derivatives or 30 mg of teicoplanin in 10 ml of CH_3CN - 0.2% aq $HCOONH_4$ (1: 1 mixture).

Retention times relative to factor A2 of teicoplanin (22.4 minutes): T-A3-1 0.41, T-A3-2 0.50, T-aglycone 0.54.

Preparation of T-Aglycone (II)

Method A: Teicoplanin (I)* (10 g) was dissolved in 200 ml of 90% aq TFA and heated to 80°C for 2 hours. After cooling to room temp, the reaction mixture was poured into ice-cooled Et_2O (1 liter). The precipitate was filtered off, washed with Et_2O and then dried in the air to yield 6.3 g of T-aglycone TFA salt.

A solution of 5.3 g of this material was dissolved in 1 liter of a mixture of 0.2% aq $HCOONH_4$ - MeOH - BuOH (1: 2: 3) and 20 g of silica gel was added. After stirring the solvents were stripped off and the residue was applied to the top of a column containing 750 g of silica gel in H_2O, developing with 2 liters of a mixture of 0.6% aq $HCOONH_4$ - CH_3CN (9: 1). The eluate was discarded then the elution was continued with a linear step-gradient of CH_3CN - H_2O from 1: 9 to 3: 7 at a rate of 200 ml/hour for 30 hours. Fractions of 25 ml each were collected and monitored by HPLC. Fractions 200 to 250 were combined and BuOH was added to prevent foam formation. The solution was concd to a small volume, Et_2O was added, and the precipitate was filtered, washed with Et_2O and dried at 40° under vacuum (yield 0.9 g, 16.9%).

Crystalline T-Aglycone: To a suspension of 0.9 g of **II** in 250 ml of a mixture of CH_3CN - H_2O (1: 9), 1 N HCl was added dropwise at room temp to pH 1.7. The resulting solution was applied to a silanized silica gel column (200 g) stabilized in 1% aq $HCOONH_4$ at a rate of 20 ml/hour. The column was eluted with 300 ml of distilled H_2O which was discarded, then it was developed with a linear step-gradient of CH_3CN in H_2O from 10% to 40% at a rate of 70 ml/hour for 30 hours. Fractions of 7 ml each were collected. Fractions 221~239 were combined. By standing for 24 hours at room temp a crystalline precipitate formed that was filtered, washed with CH_3CN (10 ml) then with Et_2O (100 ml) and recrystallized from a mixture CH_3CN - H_2O (8: 2) obtaining after drying at 2 mmHg 0.55 g of pure crystalline **II**: UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε) 279 (11,100).

Method B: To a suspension of **I** (10 g) in 80 ml of benzyl alcohol heated at 40°C 20 ml of 37% aq HCl was added with stirring. The reaction mixture was stirred under vacuum (20 mmHg) for 12 hours at 60°C while adding every one and half hour a mixture of 15 ml of benzyl alcohol and 5 ml of 37% HCl. After cooling to room temp 1 liter of Et_2O was added. The precipitate was collected, washed with Me_2CO then with Et_2O obtaining 9.2 g of crude **II**. This product was dissolved in 250 ml of a mixture of MeOH - 0.1 N HCl (3: 2) and to the resulting solution 1 liter of a mixture of H_2O - EtOAc - BuOH (4: 5: 1) was added. The pH of the aq phase was adjusted to 8.5 by adding 1 N NaOH and the organic layer was discarded. The aq phase was applied to a chromatographic column as previously described. Substantially pure **II** (1.5 g) was obtained. By treating T-A3-1 or T-A3-2 for the times indicated in Scheme 2, **II** was obtained in the same yield, respectively.

Method C: To a solution of 2.5 g of **I** in 150 ml of AcOH heated at 80°C 480 mg of Dowex HCR-S (H^+) resin containing 0.13 ml of H_2O was added. The mixture was vigorously stirred for 20 hours, the resin was filtered off, washed twice with 100 ml portions of AcOH containing 10% of 37% HCl. The combined filtrate was poured into 2 liters of Et_2O and the precipitate formed was collected, washed with Et_2O then worked up as described for Method B obtaining 0.6 g of **II**.

* Composition %: T-A3-1 5.4, factors A1 5.7, A2 30.9, A3 16.8, A4 11.6, A5 13.0, related substances 1.1, H_2O 15.0, BuOH 0.05, NaCl 0.2, Cl^- 0.25.

Method D: To a solution of 2.65 g of **I** in 180 ml of AcOH 20 ml of 37% HCl was added and the mixture was heated at 80°C for 1 hour. After cooling to room temp the reaction mixture was poured into 1 liter of ice-cooled Et_2O. The precipitate that formed was filtered off, washed with Et_2O and then dissolved in 60 ml of a mixture of MeOH - 0.1 N HCl (3: 2). The solution was worked up as described in Method B obtaining 0.75 g of **II**.

Method E: A suspension of **I** (20 g, H_2O content 12% by weight) in 300 ml of 0.5 N gaseous HCl in trifluoroethanol was heated at 80°C while continuously bubbling HCl for 12 hours. After cooling to room temp the solid formed was collected, washed with Et_2O and then suspended in 1 liter of H_2O. The pH was adjusted to 1.9 by adding 1 N HCl and the solution so obtained was washed with 1 liter of EtOAc (discarded) and then extracted with 1 liter of a mixture of BuOH - EtOAc (1: 2). The organic phase was washed with 500 ml of a satd NaCl aq solution and then concd to a small volume. By adding 500 ml of Et_2O a precipitate formed which was filtered off, washed with Et_2O and dried in the air yielding 18.6 g of crude product. It was dissolved in 1 liter of MeOH, 25 g of silanized silica gel was added and the solvent was stripped off under vacuum at 35°C. The residue was suspended in 500 ml of a mixture of 0.5% aq $HCOONH_4$ - CH_3CN (9: 1) and applied at the top of a column prepared with 2.5 kg of silanized silica gel, buffered with 1 liter of 1% aq $HCOONH_4$ and stabilized with 500 ml of the mixture 0.5% aq $HCOONH_4$ - CH_3CN (9: 1). The column was washed with 2 liters of a mixture of CH_3CN - H_2O (9: 1) and then developed with 8 liters of a linear step gradient from 10% to 40% of CH_3CN in H_2O. Fractions of 20 ml each were collected. Fractions 71 to 420 were combined and, after adding 4 liters of BuOH, concd to a small volume. By adding Et_2O 6.5 g of substantially pure **II** was obtained.

T-Aglycone Hydrochloride (**II**·HCl)

II (130 mg) was suspended in 10 ml of a mixture CH_3CN - H_2O (2: 3) and 0.2 ml of 1 N HCl was added. After adding 15 ml of BuOH the resulting solution was concentrated to 2 ml. By adding 10 ml of Et_2O a precipitate formed that was filtered, washed with Et_2O and dried under vacuum at 50°C overnight obtaining 107 mg of the title compound.

Degluco-teicoplanin Benzyl Ester, Hydrochloride (**III**)

To a solution of 10 g of **I** in 80 ml of benzyl alcohol, 20 ml of 37% HCl was added at 40°C. The mixture was kept for about 1 hour under vacuum (~20 mmHg) while heating to 60°C, then 50 ml of benzene was added and the mixture was evaporated under the same conditions. After 1 hour a mixture of 25 ml of benzyl alcohol and 5 ml of 37% HCl was added to the reaction mixture which was then re-submitted to the evaporation under vacuum.

This procedure was repeated every 30 minutes for 8 hours then 100 ml of benzene and 20 ml of 37% HCl was added. By evaporation as above a clear solution was obtained that was stirred under argon atmosphere for 12 hours at room temp. Finally, it was poured into 1.5 liters of Et_2O. A solid separated which was collected, washed with ether and dried under vacuum at room temp overnight yielding 10 g of the crude ester. This product was dissolved in 150 ml of MeOH and 300 ml of H_2O and 300 ml of EtOAc was added with vigorous stirring. After few minutes additional 300 ml of H_2O, 300 ml of EtOAc and a mixture of 300 ml of BuOH - H_2O (1: 2) was added. The pH of the aq layer was adjusted to 3.5 and the organic phase was separated. The aq phase was extracted with EtOAc (600 ml×2). The organic layers were combined, washed with 400 ml of H_2O and concd to a small volume. By adding Et_2O a solid separated which was filtered off, washed with Et_2O and dried overnight at room temp yielding 6.1 g of **III** (HPLC titer 75%, H_2O and solvents 15%, undefined impurities 10%) that was suitable for further reaction.

A highly pure sample was obtained by adding 10 g of silica gel to a solution of 2.5 g of **III** in 100 ml of 90% aq MeOH. The solvent was evaporated under vacuum and the residue was applied to a column containing 250 g of silica gel slurried in CH_3CN that was developed by sequentially using the following solvent mixture: CH_3CN (250 ml); CH_3CN - H_2O (97: 3, 500 ml); CH_3CN - H_2O (94: 6, 500 ml). The eluates were discarded, then the column was eluted with a linear gradient of CH_3CN in H_2O obtained by mixing 1.5 liters each of the solvent mixtures CH_3CN - H_2O (94: 6) and CH_3CN - H_2O (70: 30) at a rate of 200 ml/hour. Fractions of 25 ml were collected and checked by HPLC.

Fractions containing the desired compound were combined (700 ml), butanolic 0.05 M HCl was added (250 ml) and the solvents were evaporated up to a final volume of 30 ml. By adding Et_2O (300 ml) a solid separated which was collected, washed with Et_2O and dried under vacuum at 40°C for 48 hours yielding 1.6 g of pure **III**.

HPLC: t_R 1.7, relative to T-aglycone (12.1 minutes); UV λ_{max}^{MeOH} nm (ε) 282 (11,800).

Anal Calcd for $C_{65}H_{51}Cl_2N_7O_{18}\cdot HCl$: C 58.90, H 3.95, N 7.40, Cl (total) 8.02, Cl^- 2.67.
Found: C 58.57, H 4.14, N 7.28, Cl (total) 7.90, Cl^- 2.66, weight loss 10.1%.

C, H, N were determined on samples dried at 140°C under inert atmosphere; Cl values were corrected for weight loss.

T-Aglycone Hydrochloride (**II**·HCl)

A solution of 4.9 g of **III** in 500 ml of a mixture of MeOH - 0.1 N HCl (7: 3) was hydrogenated at room temp and atmospheric pressure in the presence of 3.5 g of 5% Pd on $BaSO_4$. In 30 minutes about 145 ml of H_2 were absorbed. The catalyst was filtered off and washed thoroughly with 200 ml of a solution of 50% aq MeOH. BuOH (600 ml) was added to the filtrate and washings and the mixture was concd to a small volume. By adding Me_2CO a solid separated which was collected, washed with Me_2CO then with Et_2O and dried yielding 4.0 g of essentially pure **II**·HCl.

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References

1) WILLIAMS, D. H.; V. RAJANANDA, M. P. WILLIAMSON & G. BOJESEN: The vancomycin and ristocetin group of antibiotics. *In* Topics in Antibiotic Chemistry. Vol. 5. *Ed.*, P. G. SAMMES, pp. 119~158, Ellis Horwood Ltd. Publs, Chichester, 1980
2) BARNA, J. C. J. & D. H. WILLIAMS: The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Ann. Rev. Microbiol. 38: 339~357, 1984
3) PARENTI, F.; G. BERETTA, M. BERTI & V. ARIOLI: Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. I. Description of the producer strain, fermentation studies and biological properties. J. Antibiotics 31: 276~283, 1978
4) 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
5) 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
6) HUNT, A. H.; R. M. MOLLOY, J. L. OCCOLOWITZ, G. G. MARCONI & M. DEBONO: Structure of the major glycopeptide of the teicoplanin complex. J. Am. Chem. Soc. 106: 4891~4895, 1984
7) 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. 106: 4895~4902, 1984
8) MARSHALL, F. J.: Structure studies on vancomycin. J. Med. Chem. 8: 18~22, 1965
9) DEBONO, M.; R. M. MOLLOY, M. BARNHART & D. E. DORMAN: A35512, a complex of new antibacterial antibiotics produced by *Streptomyces candidus*. II. Chemical studies on A35512B. J. Antibiotics 33: 1407~1416, 1980
10) NIELSEN, R. V.; F. HYLDIG-NIELSEN & K. JACOBSEN: Biological properties of ristocetin-ϕ-aglycone. J. Antibiotics 35: 1561~1564, 1982
11) HERRIN, T. R.; A. M. THOMAS, T. J. PERUN, J. C. MAO & S. W. FESIK: Preparation of biologically active ristocetin derivatives: Replacements of the 1'-amino group. J. Med. Chem. 28: 1371~1375, 1985
12) MCGAHREN, W. J.; R. A. LEESE, F. BARBATSCHI, G. O. MORTON, N. A. KUCK & G. A. ELLESTAD: Components and degradation compounds of the avoparcin complex. J. Antibiotics 36: 1671~1682, 1983
13) DEBONO, M.; K. E. MERKEL, R. M. MOLLOY, M. BARNHART, E. PRESTI, A. H. HUNT & R. L. HAMILL: Actaplanin, new glycopeptide antibiotics produced by *Actinoplanes missouriensis*. The isolation and preliminary chemical characterization of actaplanin. J. Antibiotics 37: 85~95, 1984

14) NAGARAIAN, R. & A. A. SHABEL (Eli Lilly): Improvements in and relating to novel glycopeptide antibiotics. Eur. Pat. Appl. 0159863, Oct. 30, 1985
15) SITRIN, R. D.; G. W. CHAN, F. CHAPIN, A. J. GIOVENELLA, S. F. GRAPPEL, P. W. JEFFS, L. PHILLIPS, K. M. SNADER & L. J. NISBET: Aridicins, novel glycopeptide antibiotics. III. Preparation, characterization, and biological activities of aglycone derivatives. J. Antibiotics 39: 68~75, 1986
16) BOECK, L. D.; F. P. MERTZ & G. M. CLEM: A41030, a complex of novel glycopeptide antibiotics produced by a strain of *Streptomyces virginiae*. Taxonomy and fermentation studies. J. Antibiotics 38: 1~8, 1985
17) HAMILL, R. L. & R. E. KASTNER (Eli Lilly): A47934 antibiotic and its production. Eur. Pat. Appl. 0100605, Feb. 15, 1984
18) HIGGINS, H. M., Jr.; M. H. MCCORMICK, K. E. MERKEL & K. H. HEINZ (Eli Lilly): Improvements in and relating to antibiotics M43A, M43B, M43C and M43D. Eur. Pat. Appl. 0159180, Oct. 23, 1985
19) ELLESTAD, G. A.; W. SWENSON & W. J. MCGAHREN: Epimerization and stereochemistry of avoparcin. J. Antibiotics 36: 1683~1690, 1983
20) BARNA, J. C. J.; D. H. WILLIAMS, P. STRAZZOLINI, A. MALABARBA & T.-W. C. LEUNG: Structure and conformation of epimers derived from the antibiotic teicoplanin. J. Antibiotics 37: 1204~1208, 1984
21) HARRIS, C. M.; H. KOPECKA & T. M. HARRIS: Vancomycin: Structure and transformation to CDP-I. J. Am. Chem. Soc. 105: 6915~6922, 1983 and references therein
22) HUNT, A. H.: Structure of the pseudoaglycon of A35512B. J. Am. Chem. Soc. 105: 4463~4468, 1983
23) FESICK, S. W.; I. M. ARMITAGE, G. A. ELLESTAD & W. J. MCGAHREN: Nuclear magnetic resonance studies on the antibiotic avoparcin. Conformational properties in relation to antibacterial activity. Mol. Pharmacol. 25: 275~280, 1984
24) HUNT, A. H.; M. DEBONO, K. E. MERKEL & M. BARNHART: Structure of the pseudoaglycon of actaplanin. J. Org. Chem. 49: 635~640, 1984
25) MCGAHREN, W. J.; J. H. MARTIN, G. O. MORTON, R. T. HARGREAVES, R. A. LEESE, F. M. LOVELL, G. A. ELLESTAD, E. O'BRIEN & J. S. E. HOLKER: Structure of avoparcin components. J. Am. Chem. Soc. 102: 1671~1684, 1980
26) MICHEL, K. H.; R. M. SHAH & R. L. HAMILL: A35512, a complex of new antibacterial antibiotics produced by *Streptomyces candidus*. I. Isolation and characterization. J. Antibiotics 33: 1397~1406, 1980
27) BONGINI, A.; J. FEENEY, M. P. WILLIAMSON & D. H. WILLIAMS: Assignment of the carbon-13-spectrum of vancomycin and its derivatives. J. Chem. Soc. Perkin Trans. II 1981: 201~206, 1981
28) WILLIAMSON, M. P. & D. H. WILLIAMS: A carbon-13 Nuclear Magnetic Resonance study of ristocetins A and B and their derivatives. J. Chem. Soc. Perkin Trans. I. 1981: 1483~1491, 1981
29) WILLIAMS, D. H.; V. RAJANANDA, G. BOJESEN & M. P. WILLIAMSON: Structure of the antibiotic ristocetin A. J. Chem. Soc. Chem. Commun. 1979: 906~908, 1979
30) HARRIS, C. M. & T. M. HARRIS: Structure of ristocetin A: configurational studies of the peptide. J. Am. Chem. Soc. 104: 363~365, 1982
31) WILLIAMSON, M. P.; D. H. WILLIAMS & S. J. HAMMOND: Interactions of vancomycin and ristocetin with peptides as a model for protein binding. Tetrahedron 40: 569~577, 1984
32) BARNA, J. C. J.; D. H. WILLIAMS & M. P. WILLIAMSON: Structural features that affect the binding of teicoplanin, ristocetin A, and their derivatives to the bacterial cell-wall model *N*-acetyl-D-alanyl-D-alanine. J. Chem. Soc. Chem. Commun. 1985: 254~256, 1985
33) WILLIAMSON, M. P. & D. H. WILLIAMS: ^{1}H NMR studies of the structure of ristocetin A and of its complexes with bacterial cell wall analogues in aqueous solution. J. Chem. Soc. Perkin Trans. I 1985: 949~956, 1985
34) ROBERTS, G. D.; S. C. CARR, S. ROTTSCHAEFER & P. W. JEFFS: Structural characterization of glycopeptide antibiotics related to vancomycin by fast atom bombardment mass spectrometry. J. Antibiotics 38: 713~720, 1985