DECHLORO TEICOPLANIN ANTIBIOTICS

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Mono- and didechlorinated derivatives of the antibiotic teicoplanin, its pseudoaglycones and aglycone, and of one amide and ester of deglucoteicoplanin were prepared under selective reductive conditions. The selectivity and rate of dehalogenation were studied and compared to those of vancomycin and deglucovancomycin.

The influence of the chlorine substituents on the mechanism of action and antibacterial activity of teicoplanin antibiotics was also investigated.

Didechlorination of teicoplanin A2, its pseudoaglycones and aglycone was carried out by catalytic hydrogenation with 10% Pd-C, whereas the selective removal of the 22-Cl was achieved with methanolic sodium borohydride-palladium chloride. The *in vitro* antibacterial activity of monodechlorinated compounds against Streptococci was comparable to that of the corresponding teicoplanin antibiotics and higher than that of didechloro derivatives. Against Staphylococci the dechlorinated compounds were generally less active than the parent teicoplanins.

One amide and one ester of deglucoteicoplanin were also mono- and didechlorinated, but dehalogenation did not substantially affect the *in vitro* activity of these derivatives in particular against Gram-positive organisms.

Though to a different extent, a certain decrease in the binding strength to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic model of the antibiotic's target peptide, was observed for all dechlorinated compounds, whose structures were determined by ^1H NMR spectroscopy, fast atom bombardment (FAB)-MS spectrometry and elemental analysis.

Chemistry

Teicoplanin[1] is a glycopeptide antibiotic belonging to the vancomycin (Van)-ristocetin family. Fig. 1 shows the structures of teicoplanin A2 complex (CTA), and of its pseudoaglycones T-A3-1 (TB) and T-A3-2 (TC), and aglycone (TD), obtained from CTA by stepwise acidic hydrolysis of the sugar moieties.[2,3]

All these compounds possess two aromatic chlorine atoms at positions 22 and 55 that were removed by catalytic hydrogenation (1 atm, 10% Pd-C, room temperature) to give didechloro derivatives (HH-CTA', HH-TB, HH-TC and HH-TD). Contrary to Van,[4] dihalogenation of teicoplanin antibiotics under these conditions proceeded unselectively to mono- and didechlorinated compounds (Scheme 1). All attempts made towards selective and stepwise dechlorination of teicoplanins with Pd catalysts, by using different hydrogenation pressures and temperatures, were unsuccessful.

The desired selectivity was achieved by reduction of CTA, TB, TC and TD with $NaBH_4$-$PdCl_2$ in

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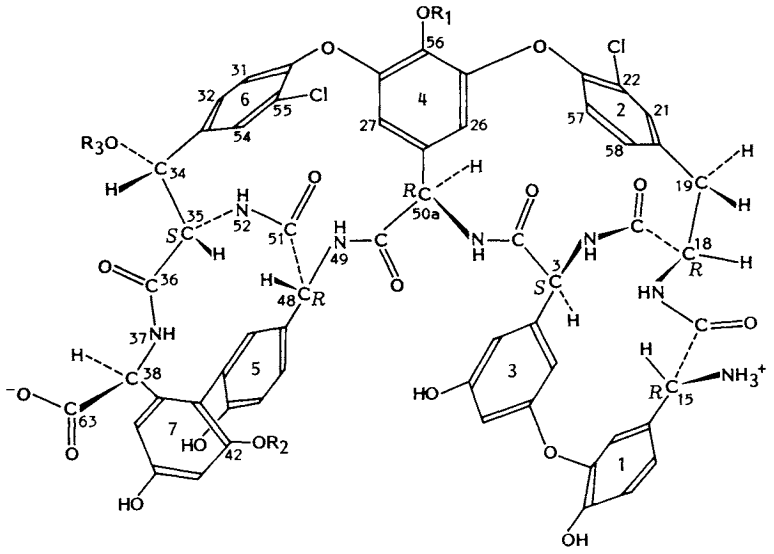
Fig. 1. Structures of teicoplanin A2 (CTA), its pseudoaglycones T-A3-1 (TB), T-A3-2 (TC), and aglycone (TD).



N-Acyl (NH-COR) side chains:

- Component A2-1 $R=(CH_2)_2CH=CH(CH_2)_4CH_3$
- Component A2-2 $R=(CH_2)_6CH(CH_3)_2$
- Component A2-3 $R=n\text{-}C_9H_{19}$
- Component A2-4 $R=(CH_2)_6CH(CH_3)CH_2CH_3$
- Component A2-5 $R=(CH_2)_7CH(CH_3)_2$

CTA R_1 = *N*-Acyl-β-D-glucosaminyl; R_2 = α-D-Mannosyl; R_3 = *N*-Acetyl-β-D-glucosaminyl

TB $R_1=H$; R_2 = α-D-Mannosyl; R_3 = *N*-Acetyl-β-D-glucosaminyl

TC $R_1=R_2=H$; R_3 = *N*-Acetyl-β-D-glucosaminyl

TD $R_1=R_2=R_3=H$

MeOH[5] (Method A, Scheme 2) which gave the corresponding 22-dechloro derivatives (H-CTA', H-TB, H-TC and H-TD) with good yields (Table 1). Reduction of the double bond of the *N*-acyl side chain of component A2-1, to give component A2-3 in CTA proceeded invariably under all the conditions.†

The conversion rate to mono- and didechloro teicoplanins was slower for CTA than for TB, TC and TD.

An important role in the reduction with $NaBH_4$-$PdCl_2$ is played by temperature which must be maintained at 30~40°C to avoid exceedingly lengthy reactions or unselective dechlorination. Different molar ratios of substrate and reagents were also investigated while maintaining the temperature at 40°C. The best results are summarized in Table 2.

Pseudoaglycones H- and HH-TB, H- and HH-TC, and aglycones H- and HH-TD were also obtained directly from H- and HH-CTA, with a few variations, under the same hydrolysis conditions described for CTA to give TB, TC and TD, respectively (Scheme 2).[2,3]

Elemental analysis and FAB-MS spectrometry indicated the presence of one chlorine atom in monodechloro teicoplanins (H-T) and the absence of chlorine in didechlorinated compounds (HH-T). Further information was obtained by ^1H NMR spectra of H-T derivatives which showed only one isomer, *i.e.* that bearing the chlorine atoms at position 55. This evidence is based on the downfield shift of the

† Component 1-free CTA (namely CTA') was also obtained before dechlorination by hydrogenation (1 atm) of CTA in the presence of 7.5% Pd-$BaSO_4$.[6]

Scheme 1.

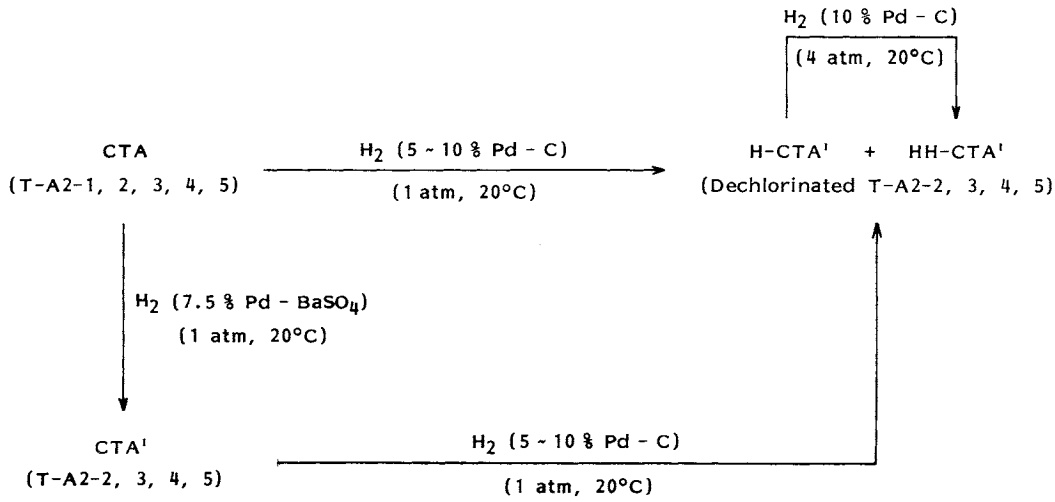


Table 1. Analytical data[a] of dechlorinated teicoplanin antibiotics.

Compound	Starting material	Method[b]	Yield (%)	HPLC[b,c] (t_R, minutes)	FAB-MS $(MH)^+$	Cl %[b] Calcd/Found	Formula[b]	MW
H-CTA'	CTA	A (C)	74 (30)	15.7[d]	ND	1.92/1.95	—	—
HH-CTA'	CTA	B	65	14.5[d]	ND	0.00/0.05	—	—
H-TB	TB	A	70	9.5	1,530	2.31/2.08	$C_{72}H_{69}N_8O_{28}Cl$	1,529.9
HH-TB	TB	B (C)	78 (37)	8.5	1,495	0.00/0.11	$C_{72}H_{70}N_8O_{28}$	1,495.4
H-TC	TC	A	50	10.5	1,368	2.59/2.46	$C_{66}H_{59}N_8O_{23}Cl$	1,367.7
HH-TC	TC	B (C)	61 (40)	10.0	1,333	0.00/0.12	$C_{66}H_{60}N_8O_{23}$	1,333.3
H-TD	TD	A	80	11.9	1,164	3.04/2.50	$C_{58}H_{46}N_7O_{18}Cl$	1,164.5
HH-TD	TD	B (C)	85 (53)	11.1	1,129	0.00/0.40	$C_{58}H_{47}N_7O_{18}$	1,130.0
H-**II**	**I, II**	A	36	18.7	1,306	2.71/2.53	$C_{65}H_{60}N_9O_{19}Cl$	1,306.7
HH-**II**	H-**II**	B	59	17.9	1,273	0.00/0.06	$C_{65}H_{61}N_9O_{19}$	1,272.3
H-**III**	**III**	A	60	11.5[e]	ND	2.90/2.83	$C_{62}H_{54}N_7O_{18}Cl$	1,220.6
HH-**III**	H-**III**	B	65	10.7[e]	ND	0.00/0.07	$C_{62}H_{55}N_7O_{18}$	1,186.2

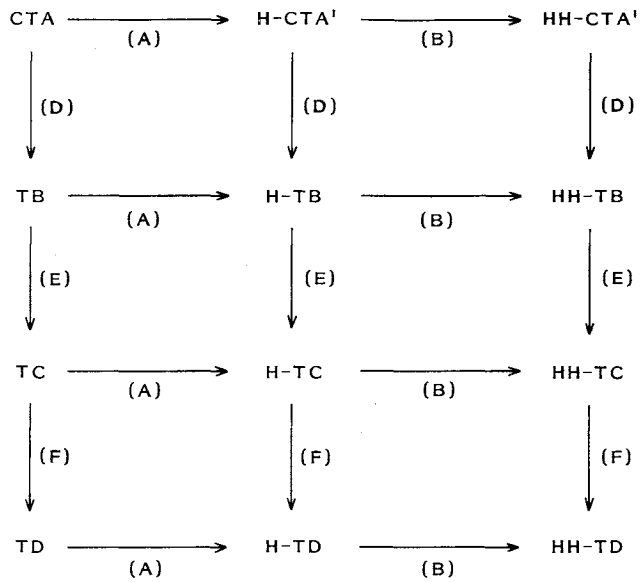
[a] Data are referred to products isolated in form of free bases or internal salts.
[b] See Experimental section.
[c] Retention time (t_R), minutes: CTA component A2-2 16.4; TB 10.4; TC 11.1; TD 12.9.
[d] Values referred to the component A2-2 of the complex.
[e] Linear step gradient from 20 to 75% of CH_3CN in 0.2% aq $HCOONH_4$ in 35 minutes at the flow rate of 2 ml/minute.
ND: Not determined.

Table 2. Conversion of teicoplanin antibiotics (T) into corresponding 22-dechloro derivatives (H-T).

T	Molar amount				
	Substrate	$PdCl_2$	$NaBH_4$	H-T[a]	HH-T[a,b]
CTA	1	20~25	400	0.9	—[c]
TB	1	20~25	350~400	0.7	0.2[d]
TC	1	15	300	0.7	0.2[d]
TD	1	7~10	140	0.9	0.1

Summary of the best conditions found at 40°C.
[a] Determined by HPLC, from the percentage of the areas of peaks.
[b] Didechloro teicoplanin antibiotics.
[c] Unidentified by-products: 10%.
[d] Unreacted material: 10%.

Scheme 2.



Methods (A) $NaBH_4$-$PdCl_2$, (B) H_2 (10% Pd-C, 4 atm), (D) 90% aq TFA, (E) HCl (DME), (F) HCl (TFE, 80°C).

Table 3. Significant ^1H NMR signals (δ, ppm) for H- and HH-TB, obtained at 40°C in DMSO-d_6.

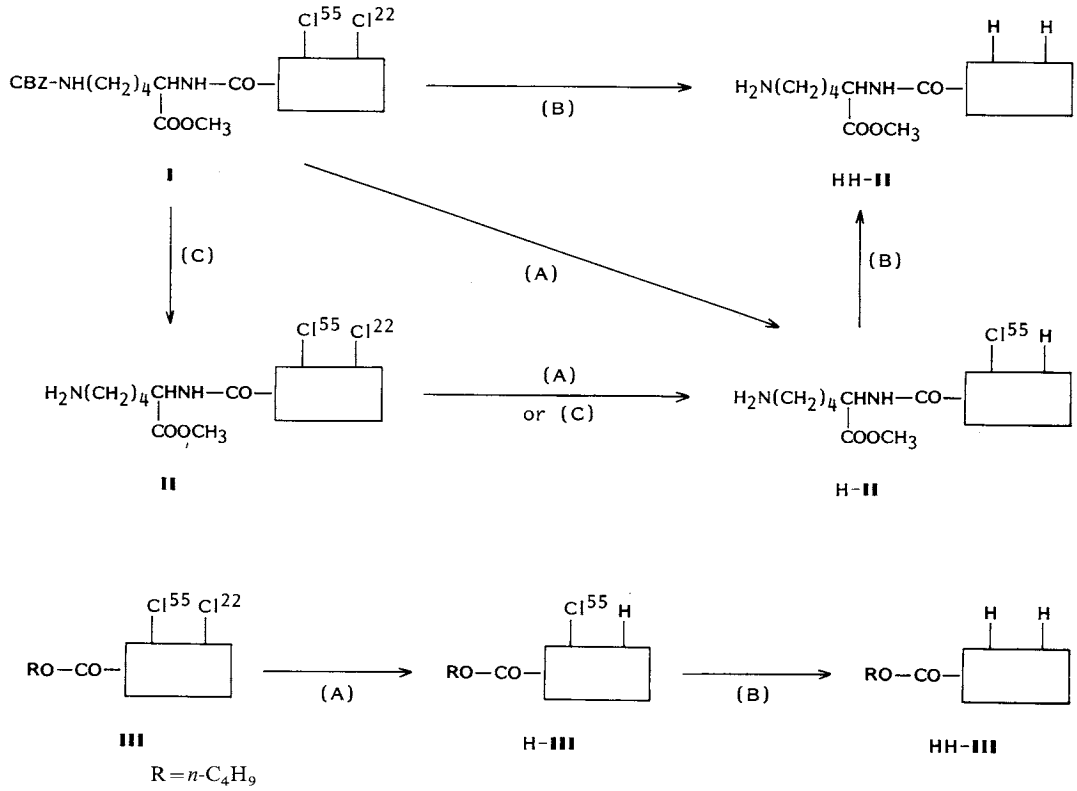
Protons[a]	H-TB	HH-TB	Protons[a]	H-TB	HH-TB
54 (6b)	7.77	7.78	48 (x5)	4.38	4.38
55 (6c)	—	6.74	38 (x7)	4.39	4.43
31 (6e)	7.23	6.81	15 (x1)	4.57	ND
32 (6f)	7.16	7.42	18 (x2)	4.56	4.59
58 (2f)	7.65	7.72	3 (x3)	5.23	5.25
57 (2e)	6.98	7.03[b]	50a (x4)	5.58	5.58
22 (2c)	7.12	7.18	26 (4b)	5.71	5.54[c]
21 (2b)	7.23	7.10[b]	27 (4f)	5.08	5.52[c]
35 (x6)	4.13	4.16	34 (z6)	5.03	5.05

[a] Between brackets, nomenclature adopted by BARNA *et al.*[7] is reported as reference.
[b,c] Might be interchanged.
ND: Not determined.

signal due to the proton at position 26, as the consequence of the deshielding resulting from a change of the relative orientation of aromatic rings 2 and 4. Likewise, in HH-T products the decreased shielding by ring 6 results in the downfield shift of the signal of 27-H. These changes in the ^1H NMR spectra were diagnostic for establishing the dechlorination position. Other modifications generally observed with respect to the ^1H NMR spectra of unmodified teicoplanin antibiotics[1~3] are the slight upfield shifts of the aromatic protons of the dechlorinated rings. Except for 18-H which is shifted slightly upfield, all the HC α resonances of the peptide backbone in H- and HH-T derivatives show the same chemical shift as in the corresponding teicoplanins. The assignments of the most significant ^1H NMR chemical shifts in H- and HH-TB are shown in Table 3; protons are designated according to both the IUPAC nomenclature and that adopted by BARNA *et al.*[7]

The feasibility of these dechlorination methods was also investigated with some derivatives of TD

Scheme 3.



Methods (A) $NaBH_4$-$PdCl_2$, (B) H_2 (10% Pd-C, 4 atm), (C) H_2 (5~10% Pd-C, 1 atm).

possessing susceptible functional groups. The 63-carboxy-amides with N_ε-benzyloxycarbonyl(CBZ)-lysine methyl ester (**I**) and lysine methyl ester (**II**), [8] and the *n*-butyl ester (**III**)[9] were selected for this purpose. As shown in Scheme 3, the ester functions were stable to the reduction that proceeded uneventfully to the corresponding mono- (H-II and H-III) and didechloro (HH-II and HH-III) derivatives, while the CBZ group was removed from compound **I** simultaneously with the displacement of the 22-Cl.

A comparative dehalogenation experiment was run between CTA and Van (Fig. 2) under the hydrogenation conditions (4 atm, 10% Pd-C) described by HARRIS *et al.*[4] The reaction course was monitored by HPLC at 4 hours intervals. CTA was completely dechlorinated to HH-CTA within 8 hours, whereas 15% Van was still present after this time and monodechloro Van (MDCV) was the only reaction product. Complete conversion of Van into MDCV was observed after 12 hours. The peak corresponding to didechloro Van (DDCV, 10%) appeared after 3 days hydrogenation. These results indicate that the dechlorination rate is faster in CTA than in Van and the displacement of both the chlorine atoms from CTA is easier than the removal of the first chlorine from Van, under the above hydrogenation conditions.

In order to establish if the different dehalogenation rate was due to a different influence of the respective sugar residues on ring 4 in CTA and Van, an additional comparative experiment was run between TD and Van aglycone (VA)[10] under milder hydrogenation conditions (3 atm, 5% Pd-C). The results are summarized in Table 4. As shown, also in this case the lability of the chlorines was higher in TD than in VA, though the reaction rate to didechloro VA (DDCVA) was faster than that observed in the

Fig. 2. Structure of vancomycin (Van).

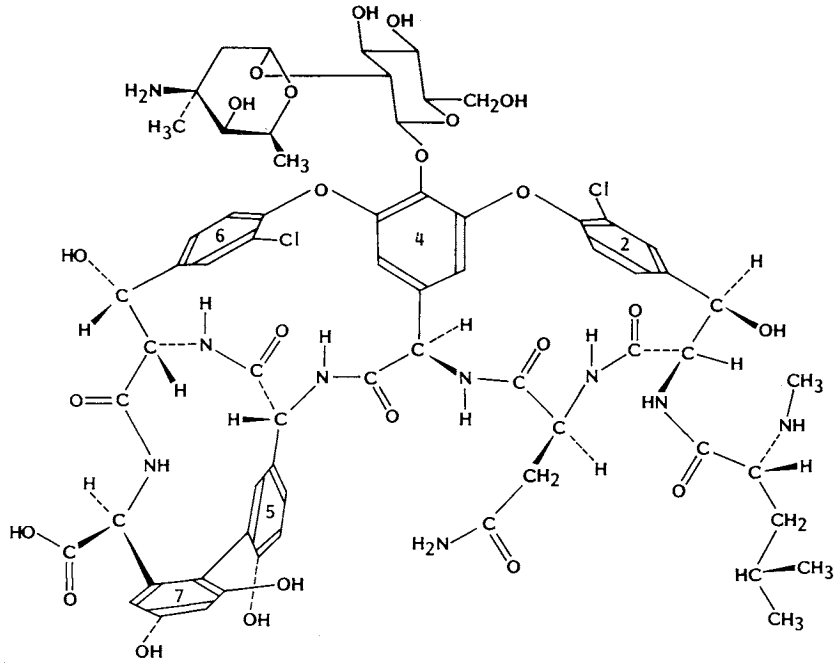


Table 4. Comparative dehalogenation rates (%) between VA and TD, under hydrogenation (5% Pd-C, 3 atm, 22°C).[a]

Time (hours)	VA	MDCVA	DDCVA	TD	H-TD	HH-TD
0	100	0	0	100	0	0
1	95	5	0	30	60	10
2	88	12	0	0	50	50
3	74	26	0	0	20	80
4	68	31	[b]	0	0	100
5	60	35	5	ND	ND	ND
10	10	50	40	ND	ND	ND
15	0	10	90	ND	ND	ND

[a] Hydrogenation was carried out under the following conditions: 1 mmol of VA or TD was dissolved in 60 ml of a MeOH-0.04 N HCl (7:3) mixture, then 1.2 g of 5% Pd-C was added and the resulting suspension was hydrogenated (3 atm) in a Parr apparatus. After 3 hours, additional 1.2 g of the same catalyst was added and hydrogenation was continued under the above conditions. The additions of fresh catalyst were repeated at times 5, 7.5, 10 and 12.5 hours.

[b] Traces.

ND: Not determined.

transformation of Van into DDCV. Moreover, for both antibiotics, the absence of the sugars on ring 4 resulted in a marked decrease in the selectivity of the dechlorination of ring 2.[†]

Peptide Binding Studies

The ability of the compounds to complex with the antibiotic's target dipeptide D-Ala-D-Ala was determined by measuring their binding to the synthetic model Ac_2-L-Lys-D-Ala-D-Ala. The differential

[†] After 4 hours hydrogenation (4 atm, 10% Pd-C), CTA was transformed into a mixture of H-CTA' (80%) and HH-CTA' (20%).

Table 5. Association constants with Ac_2-L-Lys-D-Ala-D-Ala.[a]

Compound	$K_a(M^{-1})$	Compound	$K_a(M^{-1})$
CTA	1.6×10^6	H-**II**	1.6×10^4
TB	1.2×10^6	H-**III**	3.4×10^4
TC	2.6×10^5	MDCV[b]	5.9×10^5
TD	2.5×10^5	HH-CTA	8.8×10^4
II	4.4×10^4	HH-TB	2.9×10^4
III	8.1×10^4	HH-TC	$<10^3$
Van[b]	1.5×10^6	HH-TD	$<10^3$
H-CTA	2.7×10^5	HH-**II**	$<10^3$
H-TB	7.9×10^4	HH-**III**	7.2×10^3
H-TC	3.1×10^4	DDCV[b]	1.6×10^5
H-TD	3.5×10^4		

[a] See Experimental section.
[b] Values reported by HARRIS *et al.*[4]

UV assay was used.[11]

The results (Table 5) show that the loss of the 22-Cl causes a decrease in the binding strength and the loss of both chlorines leads to a further detriment of the binding affinity. As for Van,[4] the role of the chlorine substituents in teicoplainin antibiotics is hypothesized to consist in limiting the mobility of the rings to which they are attached and thus contributing to the stability of the binding site.

Biological Activity

The loss of the chlorines in general decreased the antibacterial activity of teicoplanin antibiotics to a different extent (Table 6).

In vivo, in curing *Streptococcus pyogenes* experimental septicemia in the mouse, H-CTA′ and H-TD were about half as effective as CTA and TD, respectively. This appears to reflect their *in vitro* activity against the test strain.

The loss of the second chlorine resulted in a marked reduction of the *in vitro* activity against both Staphylococci and Streptococci. The *in vivo* efficacy of HH-TD in *S. pygenes* septicemia was consistent with its *in vitro* activity. Against Staphylococci also the activity of monodechloro derivatives was markedly reduced.

Surprisingly, amides H-, HH-**II** and esters H-, HH-**III** did not show remarkable differences with respect to the starting compounds (**II** and **III**), except for the lower activities of HH-**II** against *Streptococcus faecalis* and *Escherichia coli*.

Discussion

CTA, its pseudoaglycones (TB and TC) and TD are selectively dehalogenated at position 22 using the sodium borohydride-palladium chloride system to give the corresponding monodechloro derivatives (H-CTA′, H-TB, H-TC and H-TD) with good yields. The catalytic hydrogenation method, suitable for the preparation of MDCV and DDCV,[4] is not sufficiently selective for the monodechlorination of the teicoplanin antibiotics and VA, though it is useful in the preparation of the didechlorinated compounds (HH-CTA′, HH-TB, HH-TC, HH-TD, and DDCVA).

The selectivity of dechlorination, higher in Van than in CTA under catalytic hydrogenation conditions, depends on the difference in reactivity between the respective chlorine atoms that is higher in Van than in CTA. This is likely due to the presence on ring 4 of two sugars in Van and one in CTA, and in particular, to the higher steric hindrance exerted by vancosamine on the ring 6 of Van.[12] The shielding effect by these

Table 6. *In vitro* (MIC) and *in vivo* (ED_{50}) antibacterial activities.

Organism	MIC (μg/ml)[a]								
	CTA	H-CTA'	HH-CTA'	TB	H-TB	HH-TB	TC	H-TC	HH-TC
Staphylococcus aureus Tour	0.125	1	4	0.25	0.5	4	0.25	0.25	4
S. epidermidis ATCC 12228	0.25	2	8	0.25	2	8	0.125	0.25	8
S. haemolyticus 602[b]	4	8	ND	8	32	32	0.5	4	ND
Streptococcus pyogenes C 203[c]	0.06	0.125	0.5	0.5	1	8	0.5	1	32
S. faecalis ATCC 7080	0.125	0.25	1	2	2	16	1	2	16
Escherichia coli SKF 12140	>128	>128	>128	>128	>128	>128	>128	>128	>128
ED_{50} (mg/kg)[a] (sc)	0.12	0.31	ND	2.64	ND	ND	2.46	ND	ND

Organism	MIC (μg/ml)[a]								
	TD	H-TD	HH-TD	II	H-II	HH-II	III	H-III	HH-III
Staphylococcus aureus Tour	0.06	0.125	0.25	0.06	0.125	0.125	0.125	0.25	0.125
S. epidermidis ATCC 12228	0.016	0.125	0.5	0.06	0.06	0.125	0.06	0.06	0.125
S. haemolyticus 602[b]	0.25	1	ND	ND	ND	ND	ND	0.5	0.5
Streptococcus pyogenes C 203[c]	0.125	0.25	2	0.125	0.125	0.125	0.125	0.125	0.25
S. faecalis ATCC 7080	0.125	0.25	2	0.125	0.125	1	0.25	0.25	0.25
Escherichia coli SKF 12140	64	128	>128	4	8	32	64	64	64
ED_{50} (mg/kg)[a] (sc)	0.95	2.2	20	0.81	ND	ND	>40	>40	ND

[a] See Experimental section. [b] Clinical isolate. [c] SKF 13400.
ND: Not determined.

sugars on the chlorine on ring 6 is evident, since the 55-Cl is faster removed from TB, TC and TD than from CTA, as well as the corresponding chlorine substituent is easier displaced from VA than from Van. In any case, the Cl atom on ring 2 is removed first.

One explanation for the lower reactivity of the chlorine on ring 6 with respect to that of the chlorine on ring 2, even in the absence of the sugars on ring 4, may be that the approach by the catalyst to the Cl atom on residue 6 is still more difficult because of the orientation of this chlorine inwards the macrocyclic ring formed by amino acid fragments 4, 5 and 6.

A difference also exists between CTA and TB, TC, TD, and between Van and VA in the dechlorination rate of ring 2. The relatively lower susceptibility of the 22-Cl in CTA, compared to that of this chlorine in TB, TC, TD, is justified by the steric hindrance exerted by the *N*-acylglucosamine at the 56-position. As expected, the shielding of the 22-Cl by this sugar moiety is greater than that by glucose and vancosamine on the corresponding chlorine in Van. It results from the difference in their relative monodechlorination rates, higher between CTA and TB, TC, TD, than that between Van and VA, due to the higher shielding effect by *N*-acylglucosamine on the 22-Cl in CTA. In fact, in CTA the acyl side chain of *N*-acylglucosamine is just placed over ring 2, while in Van vancosamine is oriented towards ring 6. This is only apparently in contrast with the higher conversion rate of CTA to H-CTA' with respect to that of Van to MDCV, since also in TD the chlorine on ring 2 is faster removed than in VA.

The different mono-dechlorination rate between TD and VA is somewhat surprising, considering the structure similarities in the common aromatic portion of these aglycones. The main difference between TD and VA consists in the presence in TD of the diphenyl ether linking amino acid fragments 1 and 3, but it is quite difficult to imagine a hydrophobic interaction, between this moiety and ring 2, able to promote the displacement of the 22-Cl.

The clorine atoms in teicoplanin antibiotics and in Van seem to contribute to a similar extent in increasing the binding to Ac_2-L-Lys-D-Ala-D-Ala, in spite of the presence in teicoplanins of the diphenyl ether group which would give additional conformational stability to the binding site. This results from the comparable decrease in the affinity for the tripeptide model caused by the loss of the chlorine atoms in teicoplanins and in Van.[4]

The influence of the chlorines on the antibacterial spectrum of activity of teicoplanin antibiotics seems to depend on their position in the molecule. In particular, the 55-Cl might play a certain role in the activity against Streptococci.

The decreased binding affinity of dechloro compounds for the target peptide is almost reflected in their decreased antibiotic activity. However, though limited to one amide and one ester of TD, dechlorination does not seem to affect significantly the *in vitro* antimicrobial properties of teicoplanin when the carboxyl function is modified. In this case, the decreased binding strength caused by the loss of the chlorine substituents is likely counterbalanced by an increased ability of dechlorinated compounds to penetrate through the bacterial cell wall.

Experimental

Evaporation of solvents was carried out, after addition of BuOH to prevent foaming, with a rotary evaporator at 40 ~ 50°C under reduced pressure.

Products were purified by reverse-phase column chromatography on silanized silica gel (0.06 ~ 0.2 mm; Merck).

Reactions, column eluates and final products were checked by HPLC analyses which were performed on a column Hibar (250 × 4 mm; Merck) pre-packed with Li-Chrosorb RP-8 (10 μm), using a Varian Model 5500 LC pump equipped with a 20-μl loop injector Rheodyne Model 7125 and a Varian Model 2050 UV variable detector. Chromatograms were recorded at 254 nm. Elutions were carried out at the rate of 2 ml/minute by mixing Eluent A, 0.2% (w/v) aqueous $HCOONH_4$, with Eluent B, CH_3CN, according to a linear step gradient from 5 to 75% of Eluent B in Eluent A in 35 minutes.

Acid-base titrations were carried out under the following conditions: The sample was dissolved in methyl cellosolve (MCS) - H_2O (4 : 1), then an excess of 0.01 M HCl in the same solvent mixture was added and the resulting solution was titrated with 0.01 N NaOH.

All compounds were analyzed for C, H, N and Cl on samples previously dried at 140°C under N_2

atmosphere. The analytical results were in accordance with the theoretical values.

IR spectra (Nujol) were obtained with a Perkin-Elmer 580 spectrometer.

UV spectra were run on a Unicam SP 800 spectrometer.

^{1}H NMR spectra were recorded with a Bruker AM 250 NMR spectrometer, using TMS as the internal reference (δ 0.00 ppm).

FAB-MS positive ion spectra were recorded on a Kratos MS 50 instrument fitted with a standard FAB source and high field magnet. The samples were dispersed in a α-thioglycerol - diglycerol (1 : 1) mixture and bombarded with a 6~9 keV beam of Xe atoms.

The composition of component 1-free CTA (CTA') derivatives, expressed as the percentages of the areas of peaks (HPLC) of the components of the complex, was approximately: Factor (%) T-A2-2 (50), T-A2-3 (25), T-A2-4 (12), T-A2-5 (13).

The N^{63}-carboxyamides of TD with the N_ε-CBZ-lysine methyl ester (**I**) and lysine methyl ester (**II**), and the *n*-butyl ester (**III**) of TD were prepared as described in previous papers.[8,9]

Preparation of Component 1-Free CTA (CTA')

A solution of 2 g (about 1 mmol) of CTA sodium salt in 500 ml of a MeOH - H_2O (8 : 2) mixture was hydrogenated (1 atm, room temperature) in the presence of 2 g of 5% Pd - $BaSO_4$. After 6 hours, the catalyst was filtered off and the filtrate was concentrated to evaporate most MeOH. The resulting cloudy aqueous solution was diluted with 10 ml of H_2O and adjusted at pH 5.5 with 1 N HCl. The precipitated solid was collected by centrifugation, washed with 50 ml of H_2O and then dried *in vacuo* at 40°C overnight, yielding 1.4 g (70%) of CTA' as a mixture of hydrochloride and internal salt.

Preparation of 22-Dechloro CTA (H-CTA') (Method A)

To a stirred suspension of 10 g (about 5 mmol) of CTA, as internal salt, in 1 liter of absolute MeOH, 20 g (about 112 mmol) of $PdCl_2$ was added at room temperature under N_2 atmosphere. After 1 hour, the reaction mixture was cooled to 0~5°C and 80 g (about 2.1 mol) of $NaBH_4$ (pellets) was added portionwise in 1 hour, while maintaining the temperature below 15°C. Stirring was continued at 35~38°C for 8 hours and at room temperature overnight, afterwords the precipitated elemental Pd was filtered off and washed with 150 ml of MeOH. The filtrates were combined and the resulting solution was adjusted at pH 5 with glacial AcOH (about 120 ml), then it was concentrated to a small volume. The precipitated solid was collected and re-dissolved in 600 ml of H_2O. The resulting solution was loaded on a column of 2.5 kg of silanized silica gel in 2% (w/v) aqueous $HCOONH_4$. Desalting was carried out by eluting with 1 liter each of the following mixtures CH_3CN - H_2O: 1) 10 : 90, 2) 20 : 80, 3) 30 : 70, 4) 40 : 60, 5) 50 : 50. The product was eluted with a mixture of CH_3CN - 0.1 N AcOH (50 : 50), while collecting 500 ml fractions. Those containing the 4 components of the title compound were pooled and solvents were evaporated. The solid residue was suspended in H_2O (100 ml) and collected by filtration, washed with 50 ml of H_2O, then with 300 ml of EtOAc and dried *in vacuo* at 35°C overnight, yielding 7.3 g of H-CTA', as the internal salt.

Preparation of the 22-Dechloro Derivatives of TB (H-TB), TC (H-TC) and TD (H-TD) (Method A)

Substantially following the same procedure as described above, but starting from TB, TC and TD, respectively, the title compounds were obtained.

The only differences consisted in the reaction times, after the addition of $NaBH_4$, which were of 8, 5, and 2 hours for H-TB, H-TC, and H-TD, respectively, and temperature (40°C). Crude products thus obtained were then purified by column chromatography on 200 g of silanized silica gel (in H_2O) per 1 mmol of starting material. Elutions were carried out with a linear gradient from 10 to 60% of CH_3CN in H_2O in 12 hours at a flow rate of 300 ml/hour, while collecting 25 ml fractions.

Preparation of 22,55-Didechloro CTA (HH-CTA') (Method B)

A solution of 10 g (about 5 mmol) of CTA in 500 ml of a mixture of MeOH - H_2O (7 : 3) was adjusted at pH 7.6 with 0.01 N NaOH and hydrogenated (1 atm, room temperature) in a Parr apparatus over a period of 6 hours in the presence of 5 g of 10% Pd - C. A suspension of 10 g of the same catalyst in 200 ml of H_2O was then added and hydrogenation was continued at 4 atm for 7 hours. The dark suspension was adjusted to pH 2.5 with 1 N HCl and the catalyst was removed by filtration through a panel of 50 g of

Celite BDH-545 filter-aid. The clear filtrate was adjusted at pH 5.6 with 1 N NaOH and concentrated to a final volume of about 50 ml. On adding 100 ml of 1% (w/v) aqueous $HCOONH_4$ the precipitated solid was filtered, washed with 100 ml of H_2O and dried *in vacuo* at 40°C for 3 days, yielding 6.4 g of the title compound, as the internal salt.

Preparation of the 22,55-Didechloro Derivatives of TB (HH-TB), TC (HH-TC) and TD (HH-TD) (Method B)

Following the same procedure as described above for HH-CTA', with minor variations, a solution of 5 mmol of TB, TC or TD in 500 ml of a MeOH - 0.04 N HCl (7 : 3) mixture was hydrogenated (1 atm, room temperature) over 5 g of 10% Pd - C for 3 hours, then at 4 atm over 10 g of the same catalyst for 3, 2.5 and 2 hours, respectively. After filtration and evaporation of the solvents, the solid residue was suspended in 150 ml of 2% (w/v) aqueous $HCOONH_4$, then the insoluble matter was collected by filtration to give the title compounds, as the internal salts.

Preparation of the Mono- and Didechloro Derivatives of CTA, TB, TC and TD (Method C)

A solution of 10 mmol of the proper starting teicoplanin antibiotic (CTA, TB, TC or TD) in 1 liter of a MeOH - 0.04 N HCl (7 : 3) mixture was hydrogenated (1 atm, room temperature) in the presence of 10 g of 5% Pd - C. Within 2 hours, 220 ml of H_2 was absorbed, but no reaction occurred. After adding fresh catalyst (15 g), hydrogenation was continued until the starting material was completely transformed (HPLC) into a mixture of mono- and didechlorinated compounds (about 450 ml of H_2 was absorbed). The suspension was then filtered and the filtrate was adjusted at pH 7.2 with 1 N NaOH. Evaporation of the solvents gave a solid residue containing a mixture of mono- and didechlorinated compounds. In this mixture, the molar ratio (HPLC) between mono- and didechloro derivatives of CTA, TB, TC and TD was 75 : 25, 45 : 55, 50 : 50 and 35 : 65, respectively. The crude powder was dissolved in 1 liter of H_2O - CH_3CN (95 : 5) and chromatographed on a column of 2.5 kg of silanized silica gel in H_2O, eluting with a linear gradient from 5 to 70% of CH_3CN in 0.1 N AcOH. Fractions (25 ml each) containing the pure products were pooled and concentrated to a small volume. The precipitated solid were collected by filtration, washed with H_2O and dried *in vacuo* at 45°C for 3 days to give the title compounds, as the internal salts.

Preparation of H- and HH-TB (Method D)

A solution of 2 mmol of H- or HH-CTA' in 160 ml of 90% aqueous TFA was stirred at room temperature for 90 minutes, afterwards the solvent was evaporated and the oily residue was re-dissolved in 200 ml of a H_2O - CH_3CN (1 : 1) mixture. The resulting solution was adjusted at pH 6 with 1 N NaOH and then it was concentrated to a final volume of about 50 ml. On standing at 6°C overnight, the solid separated was collected, washed with 10 ml of H_2O and dried *in vacuo* at 30°C overnight, yielding H- (95%) or HH-TB (93%), as the internal salts.

Preparation of H- and HH-TC (Method E)

Dry HCl was bubbled at room temperature into a stirred suspension of 1 mmol of H- or HH-CTA[†] in 100 ml of 1,2-dimethoxyethane (DME) for 3 hours. The resulting solution was concentrated to a small volume (about 15 ml), then it was diluted with 200 ml of H_2O and loaded on a column of 300 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 2 to 20% of CH_3CN in H_2O in 15 hours at the flow rate of about 200 ml/hour, while collecting 15 ml fractions. Those containing pure product were combined and the solvents were evaporated to give H- (67%) or HH-TC (74%), as the hydrochlorides.

Preparation of H- and HH-TD (Method F)

A suspension of 2 mmol of H- or HH-CTA[†,††] in 200 ml of 2,2,2-trifluoroethanol (TFE) was stirred at 70°C for 6 hours, while bubbling dry HCl. The insoluble matter was collected and re-dissolved in 100 ml of a H_2O - CH_3CN (1 : 1) mixture. After adding 20 g of silanized silica gel, the resulting suspension was stirred at room temperature for 30 minutes, then it was diluted with 400 ml of H_2O, adjusted at pH 5.5

[†] H- or HH-TB, and [††] H- or HH-TC can be alternatively used.

with 1 N NaOH, and loaded on a column of 400 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 10 to 50% of CH_3CN in 0.005 N HCl in 20 hours at the rate of 200 ml/hour, while collecting 15 ml fractions. Those containing the desired product were pooled, the solvents were evaporated and the solid residue was collected, washed with Et_2O and dried *in vacuo* (over KOH) at room temperature overnight to yield H- (58%) or HH-TD (61%), as the hydrochlorides.

N^{63}-Carboxyamide of H-TD with the α-Amino Group of Lysine Methyl Ester (H-II) (Method A)

From Amide **I**: To a stirred solution of 5 g (about 3.4 mmol) of compound **I** in 800 ml of absolute MeOH, 6 g (34 mmol) of $PdCl_2$ was added at room temperature (N_2 atmosphere). After 1 hour, the reaction mixture was cooled to 0~5°C and 18 g (about 0.49 mol) of $NaBH_4$ (pellets) was added over a period of 30 minutes. The temperature rose up to 30°C and it was maintained at 35~38°C for 6 hours. After stirring at room temperature overnight, the elemental Pd was filtered off and the clear filtrate was brought to pH 5 with glacial AcOH (about 80 ml). The solvent was evaporated and the solid residue was dissolved in 300 ml of a H_2O-CH_3CN (95:5) mixture. The resulting solution was loaded on a column of 500 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 10 to 60% of CH_3CN in 0.01 N HCl in 20 hours at the rate of 200 ml/hour, while collecting 20 ml fractions. Those containing the pure product were pooled and solvents were evaporated to give 2 g of the title compound, as the dihydrochloride, which was dissolved in 200 ml of H_2O. The resulting solution was adjusted at pH 8.4 with 1 N NaOH and concentrated to a small volume (about 40 ml). On standing at 6°C overnight, a solid separated which was collected and dried *in vacuo* at room temperature overnight, yielding 1.6 g of H-**II**, as the free base.

From Amide **II**: To a stirred solution of 6 g (about 4.5 mmol) of compound **II** in 800 ml of absolute MeOH, 3.8 g (21.6 mmol) of $PdCl_2$ was added at room temperature under N_2 atmosphere. After 1 hour, the reaction mixture was cooled to 0~5°C and 12.6 g (about 0.34 mol) of $NaBH_4$ (pellets) was added over a period of 60 minutes, while maintaining the temperature below 15°C. Stirring was continued at 38°C for 4 hours, then the reaction mixture was worked up and chromatographed as above described to give 2.4 g of H-**II**, as the dihydrochloride.

N^{63}-Carboxyamide of HH-TD with the α-Amino Group of Lysine Methyl Ester (HH-II) (Method B)

A solution of 10 mmol of compound **I** (or **II**) in 1.5 liter of a MeOH - 0.01 N HCl (8:2) mixture was hydrogenated at 1 atm and room temperature in the presence of 5 g of 10% Pd-C for 2 hours, afterwards 10 g of the same catalyst was added and hydrogenation was continued at 4 atm for 6 hours. The catalyst was removed and the filtrate was adjusted at pH 8.5 with 1 N NaOH, then most MeOH was evaporated. The aqueous solution was extracted with 600 ml of BuOH. The organic layer was washed with H_2O (2 × 300 ml) and concentrated to a final volume of about 100 ml. On adding 400 ml of Et_2O the precipitated solid was collected, washed with Et_2O and dried *in vacuo* at 50°C overnight, yielding 7.4 g of the title compound, as the free base.

Preparation of Mono- (H-II) and Didechloro (HH-II) Derivatives of Amide **II** by Catalytic Hydrogenation (Method C)

A solution of 14 g (about 10 mmol) of compound **II** in 700 ml of a MeOH - 0.04 N HCl (7:3) mixture was hydrogenated (1 atm, room temperature) in the presence of 10 g of 10% Pd-C for 6 hours, while absorbing 630 ml of H_2. At this point, HPLC showed the presence of unreacted compound **II** (about 20%) and a mixture of mono- (about 45%) and didechloro (about 35%) derivatives. After adding additional 5 g of the same catalyst, hydrogenation was continued under the above conditions for 1 hour (further 240 ml of H_2 was absorbed). The catalyst was filtered off and 50 g of silanized silica gel was added to the filtrate (HPLC: Compound **II**, absent; H-**II**, 60%; HH-**II**, 40%). The solvents were evaporated and the dry residue was suspended in 300 ml of H_2O. The resulting suspension was loaded on a column of 1.4 kg of silanized silica gel in H_2O. The column was developed with a linear gradient from 10 to 50% of CH_3CN in 0.005 N HCl in 20 hours at the flow rate of 300 ml/hour, while collecting 20 ml fractions. Those containing pure products were pooled and the solvents were evaporated to yield compounds H-**II** (4.1 g) and HH-**II** (2.6 g), as the dihydrochlorides.

22-Dechloro Deglucoteicoplanin *n*-Butyl Ester (H-III) (Method A)

To a stirred solution of 3.2 g (about 2.5 mmol) of compound **III** in 300 ml of absolute MeOH, 35 g (about 200 mmol) of $PdCl_2$ was added at room temperature (N_2 atmosphere). After 45 minutes, 10 g (about 285 mmol) of $NaBH_4$ (pellets) was added portionwise over a period of 1 hour. Temperature was allowed to rise up to 30°C and it was maintained at 30~35°C for 3 hours. The HPLC profile of the reaction mixture showed the presence of compound H-**III** (about 90%) along with HH-**III** (about 10%). After adding 500 ml of MeOH and filtering off the elemental Pd, the resulting solution was adjusted at pH 5 with glacial AcOH and then concentrated to dryness. The dark brown solid residue (9 g) was dissolved in 100 ml of a MeOH - H_2O (3 : 7) mixture and the resulting solution was loaded on a column of 600 g of silanized silica gel in H_2O. Desalting was performed by eluting with 1 liter each of the following MeOH - H_2O mixtures: 1) 30 : 40, 2) 40 : 60, 3) 50 : 50, while collecting 500 ml fractions. Those containing the product were combined and the solvents were evaporated. The crude residue was chromatographed on a column of silanized silica gel (200 g), eluting with a linear gradient from 25% of CH_3CN in 0.01 N HCl to 75% of CH_3CN in 0.1 N HCl. The title compound (2 g) was thus obtained as the hydrochloride. To a stirred suspension of this product in 200 ml of H_2O, 1.5 ml of 1 N NaOH was added. The insoluble matter was collected and dried *in vacuo* at 30°C overnight, yielding 1.8 g of H-**III**, as the free base.

22,55-Didechloro Deglucoteicoplanin *n*-Butyl Ester (HH-III) (Method B)

A solution of 1 g (about 0.8 mmol) of compound H-**III** in 200 ml of a MeOH - 0.01 N HCl (9 : 1) mixture was hydrogenated at 4 atm and room temperature in the presence of 1 g of 10% Pd - C for 5 hours. The catalyst was filtered off and the solvents were evaporated. The crude solid residue was chromatographed on a column of silanized silica gel (150 g), eluting with a linear gradient from 20 to 80% of CH_3CN in H_2O. Pure compound HH-**III** was thus obtained (0.75 g) as the hydrochloride. It was then transformed into the free base (0.55 g) by treatment with 1 N NaOH, as described above for H-**III**.

Binding Assays

The interaction of Ac_2-L-Lys-D-Ala-D-Ala with teicoplanin antibiotics and their dechloro derivatives was determined by UV differential spectroscopy.[11] Experiments were run on a Perkin-Elmer 320 double-beam spectrophotometer with 4 cm pathlength not thermostated cell. The temperature was 24±2°C. The initial volume of antibiotic solution was 10 ml at 30 μM concentration in 10% MeOH in 0.02 M citrate buffer (pH 5). The difference in absorbance (ΔA) developed on adding the test peptide was monitored at wavelength (294 nm) that showed maximum change. The association constant (K_a) for complex formation of each compound was obtained from the slope of the straight line resulting from a SCATCHARD's plot, $\Delta A/(\Delta A_{max} \times C)$ vs. $\Delta A/\Delta A_{max}$, of the data. Binding constants were obtained with a standard deviation of about 20%.[13]

Determination of Antibacterial Activity

MIC was determined using microdilution method in Difco Todd-Hewitt broth (Streptococci) or Oxoid Iso-Sensitest broth (Staphylococci and Gram-negative organisms). The final inoculum was about 10^4 cfu/ml. MIC was read as the lowest concentration (expressed in μg/ml) which showed no visible growth after 18~24 hours incubation at 37°C.

Experimental septicemia was induced in groups of five mice by intraperitoneal injection of about 10^5 cells of *S. pyogenes* C 203, a challenge corresponding to about 100 times the lethal dose for 50% infected animals. Mice were treated once immediately after infection by sc administration. On the 7th-day, ED_{50} (effective dose for 50% infected animals, expressed in mg/kg), was calculated on the basis of the percentage of surviving mice at each dose by the Spearmann and Kärber method.[14]

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