SYNTHESIS AND BIOLOGICAL ACTIVITY OF DERIVATIVES OF GLYCOPEPTIDE ANTIBIOTICS EREMOMYCIN AND VANCOMYCIN NITROSATED, ACYLATED OR CARBAMOYLATED AT THE *N*-TERMINAL

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Nitrosation, carbamoylation or acylation of the glycopeptide antibiotics eremomycin or vancomycin produced series of derivatives substituted at the *N*-terminus of the peptides. Though the modified amino group in these derivatives is not capable of protonation, *N*-nitroso derivatives retain antibacterial activity *in vitro* and *in vivo*. *N*-Carbamoyleremomycin has low activity, and *N*-Cbz-eremomycin and *N*-Boc-eremomycin are devoid of antibacterial activity, both *in vitro* and *in vivo*.

Eremomycin (**I**) is a potent glycopeptide antibiotic closely related to vancomycin[1]. Glycopeptide antibiotics are considered to act on bacterial cell wall biosynthesis at the level of transpeptidation/transglycosylation by binding to peptides containing a terminal D-alanyl-D-alanyl residue, which are precursors to or the actual substrate for the transpeptidation reaction necessary for the formation of a rigid cell wall structure[2]. The chemical modifications of eremomycin carried out in these studies were directed toward broadening the antibacterial spectrum and toward enhancement of chemotherapeutic properties[3]. Elucidation of structure-activity relationships in these series is also of great importance. Recently it was shown that the affinity of eremomycin for the target site mimetic diacetyl-L-lysyl-D-alanyl-D-alanine (DALAA) was 23-fold lower than that of vancomycin while the activity of eremomycin was greater than vancomycin against staphylococci and *Bacillus subtilis* and was less affected by DALAA than that of vancomycin[4]. These data demonstrate a difference of eremomycin and indicate that structure-activity relationships found for vancomycin or teicoplanin may not be valid for eremomycin.

In this paper we study the possibility of modification of eremomycin and vancomycin with nitrosating, carbamoylating or acylating agents and the structure-activity relationships among *N*-substituted derivatives of these antibiotics (Fig. 1).

Chemistry

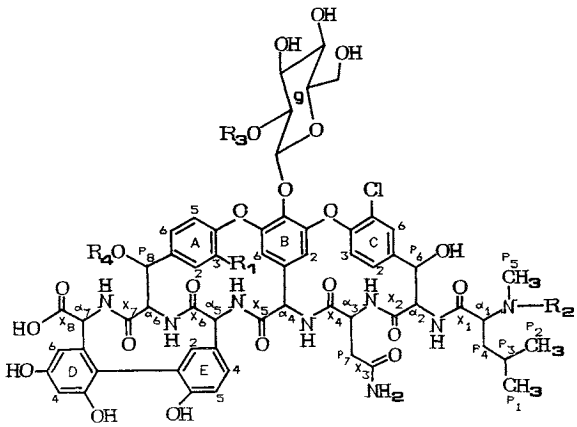
The nitrogen atom at the *N*-terminus of the peptide in eremomycin or vancomycin is designated as *N*, the nitrogen atom in the disaccharide branch of eremomycin (f fragment) or in vancomycin is designated as *N'*, and the nitrogen atom in the monosaccharide branch of eremomycin (e fragment) is designated as *N''*.

N-Nitrosated derivatives of eremomycin (**II**) (Scheme 1) and vancomycin (**X**) (Scheme 2) were prepared by the action of nitrous acid prepared *in situ* or by isoamylnitrite. The use of isoamylnitrite permitted the

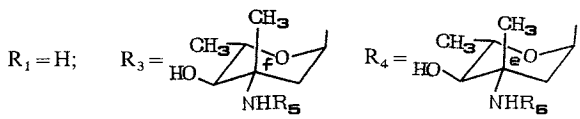
study of the nitrosation reaction in DMSO at pH 2~10. Both **I** and **IX** are nitrosated only at pH 3 the major reaction products (**II** or **X** respectively) being N–NO derivatives. The direction of electrophilic attack seems to be rather unexpected as in the molecules of **I** or **IX** there are three other groups of nucleophilic centers susceptible to nitrosation, *i.e.* amino groups of sugars, hydroxylated aromatic rings and an asparagine amido group. In our experimental conditions neither of these centers in the eremomycin or vancomycin molecule were affected, and this may be explained by the tertiary structure of the antibiotic in which some parts of the molecule are more and other less hindered. This may lead to the decreased of reactivity of some groups and enhancement of others.

Reductive alkylation of **II** or **X** with benzaldehyde and $NaBH_3CN$ gave *N'*,*N''*-dibenzyl-*N*-nitrosoeremomycin (**III**) or *N'*-benzyl-*N*-nitrosovancomycin (**XI**), respectively. Interaction of **II** with

Fig. 1. Structures of compounds **I**~**XI**.

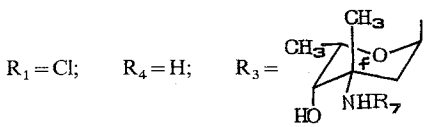


Eremomycin and its derivatives



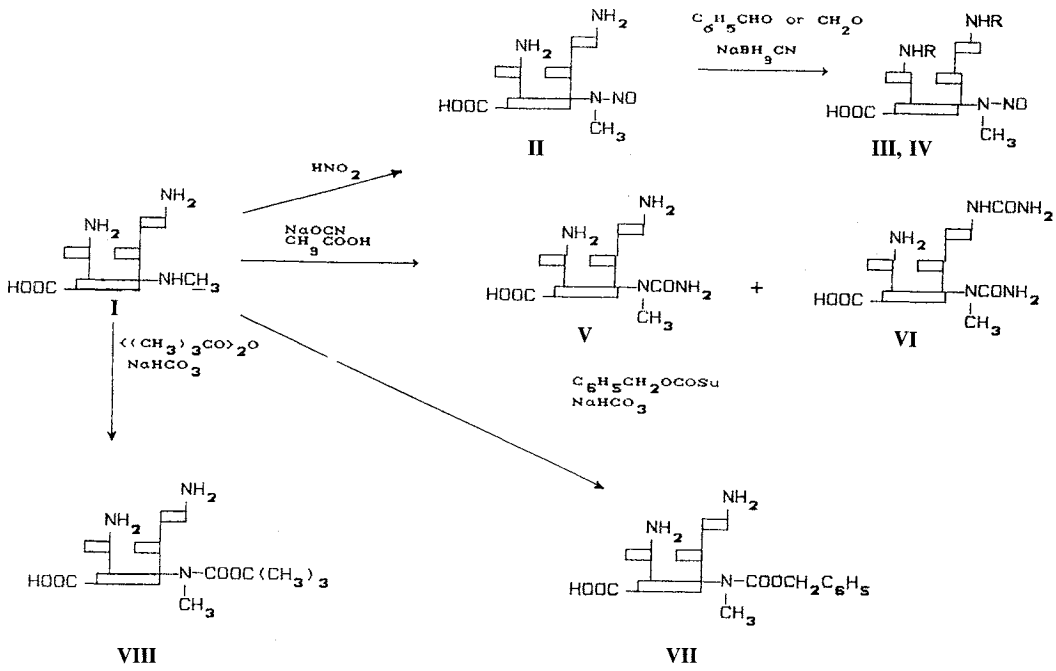
I $R_2=R_5=R_6=H$
II $R_2=NO$; $R_5=R_6=H$
III $R_2=NO$; $R_5=R_6=CH_2C_6H_5$
IV $R_2=NO$; $R_5=R_6=CH_3$
V $R_2=CONH_2$; $R_5=R_6=H$
VI $R_2=R_5=CONH_2$; $R_6=H$
VII $R_2=COOCH_2C_6H_5$; $R_5=R_6=H$
VIII $R_2=COOC(CH_3)_3$; $R_5=R_6=H$

Vancomycin and its derivatives

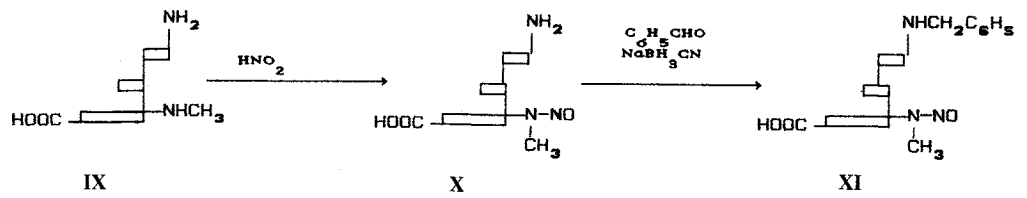


IX $R_2=R_7=H$
X $R_2=NO$; $R_7=H$
XI $R_2=NO$; $R_7=CH_2C_6H_5$

Scheme 1. Chemical transformations of eremomycin.



Scheme 2. Chemical transformations of vancomycin.



formaldehyde and reduction with $NaBH_3CN$ gave *N'*,*N''*-dimethyl-*N*-nitrosoeremomycin (**IV**).

N-Cbz-eremomycin (**VII**) and *N*-Boc-eremomycin (**VIII**) were obtained by the interaction of **I** with *N*-(benzyloxycarbonyloxy)succinimide (CbzOSu) or di-*tert*-butyl dicarbonate (Boc_2O) in water-dioxane mixture (1 : 1) in the presence of $NaHCO_3$.

N-Carbamoyl-eremomycin (**V**) and *N*,*N'*-dicarbamoyleremomycin (**VI**) were obtained by the interaction of NaNCO and **I** in the presence of acetic acid in DMSO, the directions of substitution being similar to those in the previously described acylation reaction of **I**[5].

Isolation and purification of compounds **II**, **IV**, **V**, **VI**, **VIII**, and **X** were performed by means of ion exchange chromatography on CM-cellulose similar to purification of *N*-acyleremomycins[5] and eremomycin esters[6].

Functional group electrophoretic and TLC analyses of these new derivatives are summarized in Table 1. Positions of the introduced nitroso, carbamoyl, acyl and alkyl groups were determined after acidic hydrolysis and Edman degradation. Previously it was demonstrate that hydrolysis in 0.2 N HCl at 100°C of eremomycin during 10 minutes leads to the splitting of the glycosidic bond between the two sugars in the disaccharide branch and gives rise to eremosamine[5]. TLC comparisons of the hydrolysis products

Table 1. Results of analytical investigation of compounds **I**~**XI**.

Compound	Acidic hydrolysis presence of eremosamine		Edman degradation presence of *N*-terminal *N*-methylleucine	^{1}H NMR number of introduced alkyl or acyl groups	Electrophoretic mobility relative to eremomycin	TLC Rf values	
	in disaccharide branch	in monosaccharide branch				Systems A	Systems B
I	+	+	+	—	1.00	0.30	0.05
II	+	+	−	—	0.75	0.45	0.45
III	−	−	−	2	0.70	0.55	0.52
IV	−	−	−	2	0.72	0.48	0.48
V	+	+	−	—	0.76	0.26	0.20
VI	−	+	−	—	0.55	0.29	0.50
VII	+	+	−	1	0.74	0.50	0.56
VIII			−	1	0.73	0.46	0.54
	Presence of vancoseamine						
	In disaccharide branch	Sugar is absent					
IX	+		+	—	0.75	0.40	0.07
X	+		−	—	0.54	0.52	0.49
XI	−		−	1	0.50	0.60	0.57

of compounds **III**, **IV**, **VI** with an authentic sample of eremosamine demonstrated that in these compounds the aminosugar formed is different from eremosamine. Hydolysis of **XI** led to an aminosugar different from vancosamine. Eremosamine is one of the products of hydroxysis of **II**, **V** or **VII**. Hydrolysis of **XI** gave vancosamine. TLC comparisons of the more drastic hydrolysis products of compounds **III** and **IV** (1N HCl, 100°C, 30 minutes), when glucose as well as eremosamine from the monosachharide branch are split off, with an authentic sample of eremosamine demonstrated that in these compounds the aminosugars formed are different from eremosamine. Among the drastic hydrolysis products of compounds **II**, **V**, **VI**, **VII** eremosamine was present.

Hydrolysis of **VIII** removed the Boc-group.

Edman degradation[7] showed that in all the derivatives the *N*-methylamino group is substituted: TLC demonstrated the adsence of 5-isopropyl-1-methyl-3-phenyl-2-thiohydantoin by the comparison with an authentic sample of the latter, which was obtained in a parallel experiment by Edman degradation of eremomycin.

Electrophoresis in 2N CH_3COOH demonstrated the decreased number of positively charged amino groups in compounds **II**, **V**, **VII** and **X** in comparison with the parent antibiotic and showed that the number of these groups in **III**, **IV**, **XI** is similar to the starting **II** or **X**, respectively. Compound **VI** is less positively chanrged than **V**.

The ^{1}H NMR spectra of the new compounds contained all the ^{1}H signals of eremomycin (compounds **I**~**VIII**) or vancomycin (compounds **X**, **XI**), backbone as well as those of the introduced groups (See Experimental).

The ^{13}C NMR spectra of compounds **II**, **V** and **X** are presented in Table 2. ^{13}C NMR signals were assigned by comparison with those of eremomycin and vancomycin[8].

Treatment of **I** with $Na^{15}NO_2$ in the presence of acetic acid gave ^{15}NO-eremomycin (^{15}N-**II**). The ^{15}N NMR spectrum showed two signals at 513.0 and 509.0 ppm (~3:1) in the area of *N*-nitrosoamino

Table 2. ^{13}C NMR chemical shifts (δ_C, ppm) of eremomycin (**I**), vancomycin (**IX**) and their derivatives **II**, **V** and **X**.

Assignment	**I**[a] (D_2O)	^{15}N-**II**[b] (D_2O+DMSO)	**V**[c] (D_2O+DMSO)	**IX**[a] (D_2O)	**X**[b] (D_2O+DMSO)
x-8	176.40	176.98	176.74	177.30	~177[d]
x-3	174.46	174.34	175.12	175.10	175.82
x-5	172.11	172.34	173.41	171.20	172.14
x-6	171.80	172.30	172.04	169.80	170.88
x-4	171.29	172.06	171.40	169.00	169.44
x-1	171.23	171.59	170.77	168.00	169.01
x-2	169.39	170.25	169.38	171.90	173.80
x-7	167.88	168.21	167.42	170.20	171.26
D-3	157.21	157.72	~157[d]	157.30	157.67
B-3	157.21	157.68	~157[d]	152.20	153.10
A-4	156.41	157.01[d]	156.83	149.80	149.85
D-5	155.68	156.47	155.39	155.80	156.68
E-6	155.06	155.70	154.79	155.00	155.64
B-5	153.34	153.6[d]	~153[d]	153.60	153.35
C-4	150.76	151.06	150.27	151.40	151.46
C-1	138.20	139.23	138.37	139.50	139.40
D-1	137.92	138.86	137.87	138.50	138.75
E-2	136.31	136.98	136.25	136.50	136.75
B-1	134.92	135.65	136.22	135.90	134.74
B-4	134.22	135.58	134.83	133.40	133.44
A-1	133.83	134.6[d]	~134[d]	141.20	142.11
C-6	130.70	130.79	129.69	129.60	130.18
A-2	129.42	130.03	129.06	129.10	129.10
A-6	128.79	129.47	128.89	128.70	128.84
C-5	128.35	129.15	128.70	127.50	127.45
E-4	127.60	127.79	127.24	127.30	127.24
C-2	127.28	127.18	126.10	128.10	128.33
E-3	126.56	127.12	125.91	128.10	128.33
C-3	125.56	125.73	124.86	125.30	125.18
A-3	123.55	123.83	123.18	124.70	124.53
E-1	122.17	123.06	121.91	122.20	122.83
A-5	122.09	122.75	121.81	127.10	127.08
D-2	118.27	119.12	118.15	118.50	118.56
D-6	109.09	109.33	108.65	109.10	108.61
B-2	107.71	107.9[d]	108.65	109.00	107.86
B-6	104.80	105.07	104.01	106.20	105.87
D-4	103.87	104.10	103.35	103.90	103.83
g-1	102.30	~103[d]	103.29	102.30	102.45
f-1	98.03	98.41	97.70	98.50	98.29
e-1	93.27	93.54	92.87	—	—
g-2	80.00	80.3[d]	~79[d]	80.10	79.75
g-3	77.14	77.4[d]	~77[d]	76.90	77.23
g-5	76.44	76.13	~76.5[d]	77.50	77.61
e-4	75.62	75.78	75.42	—	—
P-8	75.30	75.42	75.15	72.40	71.76
f-4	75.20	75.42	74.73	71.80	71.70
P-6	71.78	71.66	70.48	72.90	72.26
g-4	70.33	70.80	69.93	70.30	70.76
e-5	67.03	67.38	66.66	—	—
f-5	66.62	67.09	66.34	64.90	65.48
α-6	62.31	63.52	63.10	64.10	64.50
α-1	61.76	62.39	61.48	61.70	63.70
g-6	61.76	60.12	59.43	61.60	62.08
α-2	59.83	60.06	59.09	59.60	59.52

Table 2. (Continued)

Assignment	I[a] (D_2O)	^{15}N-II[b] (D_2O+DMSO)	V[c] (D_2O+DMSO)	IX[a] (D_2O)	X[b] (D_2O+DMSO)
α-7	59.46	60.06	58.50	60.40	59.90
e-3	57.45	57.84	57.14	—	—
f-3	57.11	57.43	56.66	55.60	55.46
α-5	55.34	55.87	55.14	54.90	55.13
α-4	55.21	55.57	54.88	56.00	56.18
α-3	53.10	53.44	52.61	52.50	52.30
P-4	39.76	40.14	39.37	39.60	40.38
f-2	39.40	39.56	39.70	34.00	34.00
e-2	39.08	38.37	38.25	—	—
P-7	36.95	37.12	36.25	36.50	37.16
P-5	32.66	31.13	31.72	32.90	30.77
P-3	24.53	25.41	24.86	24.90	25.30
P-1	22.78	23.56	22.64	23.10	23.46
P-2	22.03	22.00	21.12	22.80	22.02
Me-e-3	18.85	19.42	18.54	—	—
Me-f-3	18.51	19.16	18.21	22.90	23.19
Me-e-5	17.91	18.65	17.68	—	—
Me-f-5	17.59	18.30	~17[d]	17.10	17.41

[a] Data from[8].
[b] In the high field part of the spectra signals of the minor isomer of ^{15}N-II at 35.93, 31.53, 25.64, 23.41, 22.46 ppm are observed; signals of the minor isomer of X are at 36.10, 25.57, 23.27, 22.50 ppm.
[c] Signal of $CONH_2$ is observed at 162.08 ppm.
[d] Signals are broad.

compounds[9]. ^{15}N signals appeared to correspond to the *syn* and *anti* isomers of II. In the ^{13}C NMR spectra of II and X signals of minor isomers can be also seen (Table 2).

Results and Discussion

Table 3 shows the *in vitro* antibacterial activity of all synthesized compounds against *B. subtilis* and 25 clinical isolates of methicillin-resistant *Staphylococcus aureus* in comparison with the parent antibiotics I and IX. The activity of compounds II, III, X and XI against *B. subtilis* is comparable to that of vancomycin and is two fold lower than the activity of eremomycin. Compounds IV, V, VI, VII, VIII have much lower activity against *B. subtilis*. Against *S. aureus*, compounds II, III, X, and XI are 4~8 times less active than eremomycin and 2~4 times less active than vancomycin. Compounds IV, V have poor activity against *S. aureus* while compounds VI, VII and VIII are devoid of activity.

Table 3. *In vitro* activity of eremomycin (I), vancomycin (IX) and their derivatives (II~VIII, X, XI).

Compound	*B. substilis* MIC (μg/ml)	Methicillin-resistant *S. aureus* (N=25)	
		MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
I	0.08	0.5	1
II	0.16	2	4
III	0.16	4	8
IV	1.60	16	32
V	3.20	16	32
VI	4.00	>32	>32
VII	4.00	>32	>32
VIII	4.00	>32	>32
IX	0.16	1	2
X	0.16	4	8
XI	0.16	2	4

A similar response was noted when the nitroso compounds II and X were studied against greater numbers of Gram-positive clinical isolates (Table 4). Eremomycin (I) inhibited all Gram-positive isolates at a concentration <0.50 μg/ml, vancomycin (IX) at a concentration <2 μg/ml, nitroso derivatives II and

Table 4. *In vitro* activity of eremomycin (**I**), vancomycin (**IX**), *N*-nitrosoeremomycin (**II**) and *N*-nitrosovancomycin (**X**) against gram-positive clinical isolates.

Organism (No. tested)	Compound	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
S. aureus MR (8)	**I**	0.25～ 0.50	0.25	0.50
	II	1.00～ 8.00	2.00	4.00
	IX	0.50～ 2.00	1.00	2.00
	X	4.00～16.00	4.00	8.00
S. aureus MS (8)	**I**	0.12～ 0.25	0.25	0.25
	II	1.00～ 2.00	1.00	2.00
	IX	0.50～	0.50	0.50
	X	4.00～	4.00	4.00
Coagulase negative staphylococci MR (7)	**I**	0.12～ 0.50	0.25	0.50
	II	2.00～ 8.00	2.00	4.00
	IX	1.00～ 2.00	1.00	2.00
	X	4.00～16.00	8.00	8.00
Coagulase negative staphylococci MS (7)	**I**	0.12～ 0.25	0.12	0.25
	II	1.00～ 2.00	1.00	2.00
	IX	0.50～ 1.00	0.50	0.50
	X	4.00～ 8.00	4.00	8.00
Enterococcus faecium PT (9)	**I**	0.03～ 0.25	0.06	0.25
	II	0.12～ 1.00	0.50	1.00
	IX	0.12～ 0.50	0.25	0.50
	X	1.00～ 4.00	4.00	4.00
Enterococcus faecalis PT (10)	**I**	0.03～ 0.50	0.25	0.50
	II	0.50～ 4.00	1.00	4.00
	IX	0.12～ 2.00	0.25	2.00
	X	1.00～16.00	4.00	16.00

X were at least 8 fold less active (at a concentration $<8\,\mu g/ml$ for **II** and at a concentration $<16\,\mu g/ml$ for **X**). Compounds **II** and **X** like the parent antibiotics **I** and **IX** were not active against *Escherichia coli* (data not shown).

Comparison of the therapeutic effects of eremomycin and its nitroso derivative **II** against systemic staphylococcal infection in mice demonstrated that **II** was only three fold less active than **I**: ED_{50} for **I** is 2.50 mg/kg; ED_{50} for **II** is 7.50 mg/kg. Compounds **V**, **VII** and **VIII** were far less active than **I**.

Experimental

Eremomycin was produced at the Pilot Plant of the Institute of New Antibiotics, Russian Academy of Medical Sciences. All other reagents and solvents used were commercial products. ^{15}N $NaNO_2$ (95% isotopic enrichment) was obtained from ISOTOP (USSR). All NMR measurements were obtained with a Varian VXR-400 instrument operated at 400 MHz for ^1H, at 100.6 MHz for ^{13}C and at 40.5 MHz for ^{15}N. ^1H NMR spectra were recorded at 80°C, ^{13}C NMR spectra were obtained at 70°C using signals of solvent (DMSO-d_6) as an internal standard (δ 39.5 ppm). ^{15}N NMR spectra were recorded at 70°C with gated decoupling. ^{15}N chemical shifts were measured from NH_4 (δ 0 ppm) as an external standard.

CM-cellulose column chromatography was performed on a LKB Ultragrad Gradient Mixer 11300, supplied with Uvicord 2138 and Recorder 2065. Precoated Merck Silica gel $60F_{254}$ plates were used for TLC in systems: ProOH - EtOAc - conc. NH_4OH, 3 : 3 : 4 (A) and BuOH - AcOH - H_2O, 5 : 2 : 3 (B). Paper electrophoresis was carried out in 2 N AcOH at 700 V for 3 hours. Ion-exchange resin SDW-3, an analog of Dowex 50X2, was obtained from BioLar, Olaine, Latvia.

N-Nitrosoeremomycin (**II**)

A. Isoamyl nitrite (0.067 ml, 0.5 mmol) and 0.5 ml of AcOH were added to a stirred solution of

eremomycin sulfate (330 mg, 0.2 mmol) in 15 ml of DMSO. The reaction mixture was stirred at room temperature for 5 hours. After addition of 200 ml of acetone the precipitated solid was collected by filtration and purified by CM-cellulose column chromatography (column 1 × 40 cm) on CM-32 (Whatman) using 0.02 M $AcONH_4$ - 0.2 M $AcONH_4$ gradient (8 hours). The solution was adjusted to pH 2, passed through ion exchange column (0.5 × 6 cm) of SDW-3 (H^+) and eluted with 0.25 N NH_4OH. After concentration of the eluate to 3 ml and neutralization to pH 6 with 6 N H_2SO_4 100 ml of acetone was added to give a precipitate of **II** (210 mg, 61%).

B. $NaNO_2$ (14 mg, 0.2 mmol) and 0.1 ml of AcOH were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 10 ml of H_2O - DMSO (4 : 1). The reaction mixture was stirred at room temperature for 0.5 hour. Isolation and purification of **II** was performed as described above.

N-Nitrosovancomycin (**X**)

A. **X** (185 mg, 61%) was obtained from 297 mg (0.2 mmol) of vancomycin hydrochloride and 0.067 ml (0.5 mmol) of isoamyl nitrite by a method similar to that described for **II**.

B. **X** (145 mg, 48%) was obtained from vancomycin hydrochloride (297 mg, 0.2 mmol) and 14 mg (0.2 mmol) of $NaNO_2$ by the method similar to that described for **II**.

N',*N''*-Dibenzyl-*N*-nitrosoeremomycin (**III**)

To a stirred solution of **II** (168 mg, 0.1 mmol) in 8 ml of DMSO, C_6H_5CHO (0.061 ml, 0.6 mmol) and $NaBH_3CN$ (25 mg, 0.4 mmol) were added. The reaction mixture was stirred at room temperature for 6 hours and a product was precipitated with 150 ml of Et_2O. It was collected by filtration and redissolved in 90 ml of a mixture of BuOH - H_2O (2 : 1). The resulting solution was washed with H_2O (60 ml × 2), the organic layer was separated and concentrated to a small volume (~2 ml). After addition of 100 ml of Et_2O the precipitate was collected by filtration to yield 140 mg (75%) of **III**.

^1H NMR (CD_3OD): δ 7.3~7.6 ppm (10H, m, 2 × C_6H_5).

N'-Benzyl-*N*-nitrosovancomycin (**XI**)

Starting from **X** (151 mg, 0.1 mmol) and using substantially the same alkylation procedure described for **III** 125 mg (78%) of the pure title compound was obtained.

^1H NMR (CD_3OD): δ 7.3~7.6 ppm (5H, m, C_6H_5).

N',*N''*-Dimethyl-*N*-nitrosoeremomycin (**IV**)

To a stirred solution of **II** (168 mg, 0.1 mmol) in 8 ml of a mixture of H_2O - DMSO (1 : 1), a 37% (w/v) water solution of formaldehyde (0.075 ml, 1 mmol) and 50 mg (0.8 mmol) of $NaBH_3CN$ were added. The reaction mixture was stirred at room temperature for 4 hours and was precipitated with 100 ml of acetone. The purification was performed as described for **II** to give 90 mg (53%) of **IV**.

^1H NMR (D_2O - DMSO, 2 : 1): δ 3.0 ppm (6H, s, CH_3 × 2).

N-Carbamoyleremomycin (**V**) and *N*,*N''*-dicarbamoyleremomycin (**VI**)

NaNCO (20 mg, 0.3 mmol) and AcOH (0.6 ml) were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 15 ml of DMSO. After 15 hours 150 ml of acetone was added to give a precipitate of a mixture of **V** and **VI**, which were separated and purified by the method described for **II** to yield 160 mg (47%) of **V** and 80 mg (23%) of **VI**.

N-Carbobenzoxyeremomycin (**VII**)

CbzOSu (100 ml, 0.4 mmol) and $NaHCO_3$ (17 mg, 0.2 mmol) were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 10 ml of H_2O - dioxane mixture (1 : 1) at room temperature. After 10 hours 150 ml of acetone was added. Purification of the precipitate was performed as described for **III** to give 150 mg (42%) of **VII**.

^1H NMR (D_2O - DMSO, 2 : 1): δ 7.3~7.6 ppm (5H, m, C_6H_5).

N-Boc-eremomycin (**VIII**)

Boc_2O (109 mg, 0.5 mmol) and $NaHCO_3$ (17 mg, 0.2 mmol) were added to a stirred solution of

eremomycin sulfate (330 mg, 0.2 mmol) in a mixture of H_2O-dioxane (1:1) at room temperature. After 8 hours 150 ml of acetone was added. Purification of the precipitate was performed as described for **II** to give 140 mg (40%) of **VIII**.

^1H NMR (D_2O-DMSO, 2:1): δ 0.9 ppm (9H, s, CMe_3).

In vitro antibacterial activity against *Bacillus subtilis* ATCC 6633 was determined by the microdilution method in tubes in medium containing: 1,000 ml of Hottinger hydrolysate with 28 mg% of amine N, 30 g of agar, 5 g of peptone and 2.5 g of $CaCO_3$. Sterilization was performed at pH 7.2~7.4. Activity of the compounds against 25 methicillin resistant clinical isolates of *Staphylococcus aureus* was determined by the agar dilution method. Agar plates containing 15 ml of agar containing two-fold serial dilutions of the test agents were inoculated to yield a final density of approximetely 10^7 CFU per spot. The plates were incubated at 35°C for 24 hours in ambient air.

Minimal inhibitory concentrations (MIC's) of **I**, **II**, **IX**, and **X** against an expanded spectrum of Gram-positive bacteria (Table 4) were determined by the microdilution method as recommended by the National Committee for Clinical Laboratory Standards[10]. The tests were performed in 96 well microtiter plates using Mueller-Hinton II broth with a final volume of 0.1 ml per well and an inoculum density of approximately 10^5 CFU/ml. The medium was supplemented with 5% sheep blood cells when testing non enterococcal streptococci. Plates were incubated at 35°C for 18~24 hours in ambient air. MIC values were defined as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.

Antibacterial activity *in vivo*. The therapeutical effect of **I** and **II** were determined against acute lethal staphylococcal infection in albino mice. Mice (strain BDF_1 (20 ± 2 g)) were challenged by intraperitoneal injections with sufficient bacterial cells suspended in hog gastric mucin to kill nontreated control within 24~48 hours. The test compounds were administered subcutaneously 30 minutes and 24 hours post infection. In each test 5 mice were treated per one dose level. On the 7th day ED_{50} were calculated on the basis of the percentage of surviving mice at each dose using Berens method.

References

1) GAUZE, G. F.; M. G. BRAZHNIKOVA, N. N. LOMAKINA, T. F. BERDNIKOVA, G. B. FEDOROVA, N. L. TOKAREVA, V. N. BORISOVA & G. Y. BATTA: Eremomycin—new glycopeptide antibiotic: Chemical properties and structure. J. Antibiotics 42: 1790~1799, 1989
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. & B. CAVALLERI: Novel glycopeptide antibiotics of the dalbaheptide group. Drugs of the future 15: 57~72, 1990
4) GOOD, V. M.; M. N. GWYNN & D. J. C. KNOWLES: MM45289, a potent glycopeptide antibiotic which interacts weakly with diacetyl-L-lysyl-D-alanyl-D-alanine. J. Antibiotics 43: 550~555, 1990
5) OLSUFYEVA, E. N.; T. F. BERDNIKOVA, N. Y. DOKSHINA, N. N. LOMAKINA, G. I. ORLOVA, I. V. MALKOVA & I. N. PROZOROVA: Eremomycin modification at amino groups. Antibiotiki i Khimiotherapia (Russ) 34: 352~358, 1989
6) PAVLOV, A. Y.; E. N. OLSUFYEVA, T. F. BERDNIKOVA, B. V. ROSYNOV, L. G. ALEKSANDROVA, I. V. MALKOVA & M. N. PREOBRAZHENSKAYA: Synthesis and antibacterial activity of the antibiotic eremomycin esters. Bioorg. Khimia (Russ.) 17: 849~854, 1991
7) BERDNIKOVA, T. F.; N. L. TOKAREVA, E. A. ABRAMOVA, N. Y. DOKSHINA, N. P. POTAPOVA & N. N. LOMAKINA: A novel polycyclic glycopeptide antibiotic eremomycin. Structure of the aglycone. Antibiotiki i Khimiotherapia (Russ.) 33: 566~570, 1988
8) BATTA, G. Y.; F. SZTARICSKAI, K. E. KÖVÉR, C. RÜDEL & T. F. BERDNIKOVA: An NMR study of eremomycin and its derivatives. Full ^1H and ^{13}C assignment, motional behavior, dimerization and complexation with Ac-D-Ala-D-Ala. J. Antibiotics 44: 1208~1221, 1991
9) MARTIN G. J.; M. L. MARTIN & J.-P. GOUESHARD: ^{15}N NMR Spectroscopy *In* NMR Basic Principles and Progress. *Eds.* P. DIEHL, E. FLUCK & R. KOSFELD, p. 382, Springer Verlag, Berlin-Heidelberg-New York, Vol. 18, 1981
10) National Committee for Clinical Laboratory Standards 1985. Methods for dilutions antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard MF-A. National Committee for Clinical Laboratory Standards, Villanova, Pa