MODIFICATION OF GLYCOPEPTIDE ANTIBIOTIC EREMOMYCIN BY THE ACTION OF ALKYL HALIDES AND STUDY ON ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS OBTAINED

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(Received for publication October 13, 1993)

Alkylation of glycopeptide antibiotic eremomycin by the action of different alkyl halides leads, depending on the structure of alkyl halides used, to eremomycin derivatives of six types; alkylated at the *N*-terminus, quaternary compounds at the *N*-terminus, eremomycin esters, esters of eremocycin alkylated at the *N*-terminus, esters of eremomycin quaternised at the *N*-terminus, esters of eremomycin alkylated both at the *N*-terminus and at the aminogroup of disaccharide branch. Five compounds demonstrated high antibacterial activity *in vitro*, *N*-allyleremomycin and methyl ester of *N*,*N*-dimethyleremomycin being at least as good as the parent eremomycin.

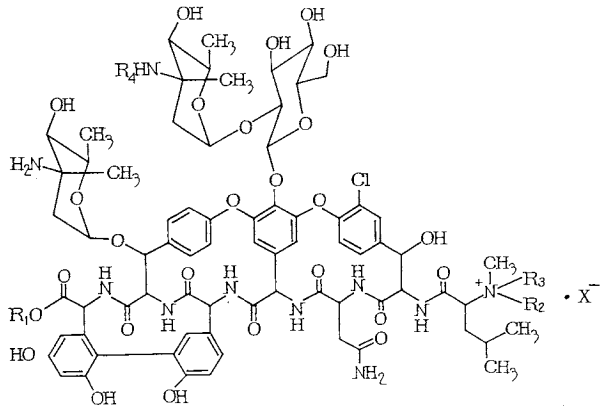
Eremomycin[1] is a glycopeptide antibiotic of dalbaheptide group[2] identical to antibiotic A82846A[3] and antibiotic MM45289[4]. It is suitable for treatment of severe Gram-positive infections and is now under preparation for clinical trial in Russia. The mechanism of action of eremomycin is similar to that of other glycopeptide antibiotics: it binds to the terminal D-alanyl-D-alanine of the muramylpentapeptide during the biosynthesis of bacterial cell wall peptidoglycan[5].

The nitrogen atom at the *N*-terminus of the peptide in eremomycin (Ere) is designated as N, the nitrogen atom in disaccharide branch as N′, and the nitrogen atom in monosacchride branch is designated as N″. Recently a series of different *N*-alkylderivatives was obtained by the reductive alkylation of Ere[6,7]. During the process of reductive alkylation, aldehydes in the presence of $NaBH_3CN$ first interact with the $N'H_2$ group in disaccharide branch of Ere, then with the $NHCH_3$ group; the reactivity of the $N''H_2$ group in this reaction is the lowest. Acylation of Ere led first to *N*-acylderivatives, *N*′-acylated compounds being isolated as minor reaction products[6,7]. Different esters of Ere were obtained by the interaction of the antibiotic with diazoalkanes[8].

In our studies on eremomycin, our attention was focused on the relationship between the level and type of alkylation of amino moieties, especially at the *N*-terminus of the antibiotic and the antibacterial activity. The influence of the transformation of COOH group at the *C*-terminus into an ester on the antibacterial activity of these alkylated eremomycins was also investigated. Herein, we wish to report the chemical transformation of eremomycin by the action of alkyl and arylalkyl halides.

Chemistry

A series of alkyl derivatives of eremomycin was prepared by the interaction of Ere with alkyl or



Type I:	**Ib**	$R_1=R_2=R_4=H$; $R_3=CH_2CH=CH_2$
Type II:	**IIa**	$R_1=R_4=H$; $R_2=R_3=CH_3$
	IIb	$R_1=R_4=H$; $R_2=R_3=CH_2CH=CH_2$
Type III:	**IIId**	$R_1=n\text{-}C_3H_7$; $R_2=R_3=R_4=H$
	IIIe	$R_1=n\text{-}C_7H_{15}$; $R_2=R_3=R_4=H$
	IIIf	$R_1=n\text{-}C_{12}H_{15}$; $R_2=R_3=R_4=H$
	IIIg	$R_1=n\text{-}C_{18}H_{37}$; $R_2=R_3=R_4=H$
Type IV:	**IVc**	$R_1=R_2=CH_2C_6H_5$; $R_3=R_4=H$
	IVd	$R_1=R_2=n\text{-}C_3H_7$; $R_3=R_4=H$
	IVe	$R_1=R_2=n\text{-}C_7H_{15}$; $R_3=R_4=H$
Type V:	**Va**	$R_1=R_2=R_3=CH_3$; $R_4=H$
Type VI:	**VIc**	$R_1=R_2=R_4=CH_2C_6H_5$; $R_3=H$
	VId	$R_1=R_2=R_4=n\text{-}C_3H_7$; $R_3=H$
	VIe	$R_1=R_2=R_4=n\text{-}C_7H_{15}$; $R_3=H$

arylalkyl halides in DMSO in the presence of $NaHCO_3$. Depending on the nature of alkylating agents, six types of Ere derivatives were obtained:

type I—*N*-alkyl derivative **Ib**,

type II—quaternary *N*-derivatives (**IIa**, **IIb**),

type III—esters of eremomycin (**IIId**, **IIIe**, **IIIf**, **IIIg**),

type IV—esters of *N*-alkly derivatives (**IVc**, **IVd**, **IVe**),

type V—methyl ester of quaternary derivative **Va**,

type VI—esters of *N,N'*-dialkyl derivatives (**VIc**, **VId**, **VIe**).

The most reactive alkyl halides (CH_3I or $CH_2=CHCH_2I$) attack first the $NHCH_3$ group of the peptide to give the compounds of types I and II, and then the carboxy group is alkylated. This sequence of events is evident if reaction is monitored by TLC. Thus, by the interaction of Ere with methyl iodide, quaternary salt **IIa** and its methyl ester **Va** were isolated with yields of 45% and 20%, respectively. In similar conditions, interaction of Ere with allyl iodide gave a mixture of products with *N*-allyleremomycin (**Ib**) as a major product (shown by TLC). Increasing the quantities of allyl iodide and $NaHCO_3$, as well as the reaction time, led to the formation of two products: **Ib** (35%) and its quaternary salt **IIb** (25%).

In contrast to methyl iodide and allyl iodide, interaction of benzyl chloride with Ere did not produce quaternized derivatives. In this case, benzyl esters of *N*-benzyleremomycin (**IVc**) and of *N,N'*-dibenzyl-eremomycin (**VIc**) were isolated in yields of 20% and 12%, respectively. It suggests that the absence of quaternized compounds among reaction products in this case is the result of steric hindrance displayed

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

Compound	Acidic hydrolysis presence of eremosamine		Edman degradation (presence of *N*-methyl-leucine at the *N*-end)	^{1}H NMR data (number of introduced alkyl groups)	IR spectroscopy data (presence of ν_{CO} at 1750 cm^{-1}) (COOR)	$[\alpha]_D^{22}$ (Cl) (Solvent)
	in disaccharide branch	in monosaccharide branch				
Ere	+	+	+	–	–	−100° (H_2O)
Ib	+	+	–	1	–	−101° (H_2O)
IIa	+	+	–	2	–	−104° (H_2O)
IIb	+	+	–	2	–	−103° (H_2O)
IIId	+	+	+	1	+	− 94° (H_2O)
IIIe	+	+	+	1	+	− 96° (MeOH)
IIIf	+	+	+	1	+	− 97° (MeOH)
IIIg	+	+	+	1	+	− 92° (MeOH)
IVc	+	+	–	2	+	−100° (MeOH)
IVd	+	+	–	2	+	− 96° (H_2O)
IVe	+	+	–	2	+	− 94° (MeOH)
Va	+	+	–	3	+	−103° (H_2O)
VIc	–	+	–	3	+	−100° (MeOH)
VId	–	+	–	3	+	− 99° (H_2O)
VIe	–	+	–	3	+	−101° (H_2O)

by the bulky benzyl group.

As in the acylation of eremomycin, the reaction of alkyl halides with the $NHCH_3$ group was more reactive than the $N'H_2$ amino group of disaccharide moiety.

Less reactive propyl iodide and heptyl iodide first attack the carboxy group to yield corresponding esters, then the $NHCH_3$ group at the *N*-terminus and finally the $N'H_2$ group. By the interaction of Ere with PrI, the propyl ester of eremomycin (**IIId**), the propyl ester of *N*-propyleremomycin (**IVd**) and the propyl ester of *N*,*N'*-dipropyleremomycin (**VId**) were isolated with the yields 17%, 13% and 6%, respectively.

Similarly, heptyl iodide yields heptyl esters of Ere (**IIIe**), of *N*-heptyleremomycin (**IVe**), and of *N*,*N'*-diheptyleremomycin (**VIe**) with the yields 18%, 16% and 12%, respectively.

Interaction of Ere with dodecyl or octadecyl bromides also produced mixtures of products of types **III**, **IV** and **VI** as it was demonstrated by TLC. We used the reaction conditions (40~45°C and 4~8 hours) which permitted the production of only esters (type III compounds), which were separated from the starting Ere by extraction with butanol. Using this approach, heptyl, dodecyl and octadecyl esters of eremomycin (**IIIe**, **IIIf** and **IIIg**) were isolated in the yields 15%, 15% and 13%, respectively.

Purification of the compounds obtained was performed with the use of ion-exchange chromatography on CM-cellulose by the method which was successfully used for the purification of the methyl ester of eremomycin[8]. The lipophilic compounds **IVc**, **VIc**, **IVe**, **VIe**, which were insoluble in water, were purified by preparative TLC.

Analytical investigation of the compounds obtained is summarized in the Table 1. Positions of the introduced alkyl groups were determined after acidic hydrolysis and Edman degradation. Previously it was demonstrated that hydrolysis in 0.2 N HCl at 100°C of eremomycin during 10 minutes leads to the splitting of the glycosidic bond between two sugars in the disaccharide branch and gives rise to eremosamine[6]. Comparison by TLC of hydrolysis products of compounds **I**~**VI** with the authentic sample of eremosamine demonstrated that among products of hydrolysis of **I**~**V**, eremosamine is present; while

aminosugars formed after hydrolysis of compounds **VI** are different from eremosamine. Hydrolysis in more drastic conditions (1 N HCl, 100°C, 30 minutes), when glucose as well as eremosamine from monosaccharide branch are released, gave for all the compounds obtained (**I**~**VI**) eremosamine.

Edman degradation[9] confirmed that in all the compounds **I**, **II**, **IV**, **V**, **VI**, the *N*-methylaminogroup is substituted: TLC demonstrated the absence 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. In eremomycin esters (type **III**) $NHCH_3$ group is not affected.

Presence of ester groups in the compounds **III**, **IV**, and **VI** is well demonstrated by IR spectroscopy.

In ^1H NMR spectra of the compounds obtained, all signals of protons of the eremomycin backbone and of the introduced groups are represented. Comparison of ^1H NMR spectra of **IIa** with Ere demonstrates the presence of a nine proton singlet at 3.2 ppm which corresponds to three *N*-methyl groups; in the ^1H NMR spectrum of **Va**, additionally to this nine proton singlet of three *N*-methyl groups at 3.20 ppm a singlet of COOMe group at 3.80 ppm is present. In ^1H NMR spectra of allyl derivatives **Ib** and **IIb**, multiplets at 6.0~5.5 ppm are present, and benzyl derivatives demonstrate multiplets in low field at 7.6~7.3 ppm. In ^1H NMR spectra of propyl, heptyl, dodecyl and octadecyl compounds, signals of terminal aliphatic CH_3 group of the introduced fatty acid substituents at 0.8 ppm and signals of CH_2 groups (except those attached to a heteroatom) can be identified at 1.0~1.2 ppm.

For the most biologically active compounds (**Ib**, **IIa**, **IIb**, **IIId** and **Va**), HPLC demonstrated that the amount of the parent Ere is lower than 1%.

Analysis of New Glycopeptides by Fast Atom Bombardment (FAB) and Ionspray Mass Spectroscopy

FAB and ionspray (pneumatically assisted electrospray) mass spectroscopy has provided molecular weight information of five glycopeptide samples (**Ib**, **IIa**, **IIb**, **IIId** and **Va**). The signals observed by FAB were very weak and several scans were accumulated to obtain reasonable spectra. The FAB analysis was slow, each sample required 30 to 40 minutes analysis time. The ionspray spectra were most intense and showed doubly charged ions as major ions in each spectrum. Weak $(M+H)^+$ ions were detected and significant fragment ions corresponding to successive losses of sugar groups were also observed. The ionspray data was obtained by a rapid flow injection technique and each sample was analyzed within 5 minutes. The FAB mass spectra of compounds **IIa**, **IIb**, **IIId**, **Va** all confirm the expected molecular weights (1,584, 1,636, 1,598, 1,598, respectively). The spectrum of **Ib** indicates a MW of 1,598, 2amu higher than expected for *N*-allyleremomycin. For mass spectrometric investigation we took a sample which previously was used for NMR study in D_2O solution; as ^1H-^2H exchange is rather slow in eremomycin derivatives (more than 10 hours) mass-spectrum of **Ia** corresponds to dideutero derivative of **Ia**. The FAB spectrum of **IIb** shows a weak $(M+H)^+$ cluster confirming the expected MW of 1636, but the most abundant peak cluster is due to addition of thioglycerol from the FAB matrix.

The positive ion ionspray spectra were obtained for compounds **Ib**, **IIa**, **IIb**, **IIId** and **Va**. $(M+H)^+$ peaks are very small in all five spectra. However, the presence of intense doubly charged peaks $(M+2H)^{2+}$ (794.2, 819.8, 800.8 and 800.8 for **IIa**, **IIb**, **IIId** and **Va**, respectively) provides the required molecular weight information. Again **Ib** shows a doubly charged species at 800.3 indicating a MW of 1598 which corresponds to a dideuterated compound.

The ionspray spectra show fragment ions indicating common losses from all 5 glycopeptides. The

spectra show 2 successive losses of 142 μ followed by a further loss of 162 μ. The FAB spectra generally show a loss of 142 μ followed by a loss of 162 μ but the signals were very weak (signal: noise typically 3 : 1). These fragmentations are due to losses of the sugars eremosamine (142) and glucose (162).

The results of these experiments clearly show that ionspray ionisation is the most efficient technique for the analysis of glycopeptides. Ionspray provides more intense spectra than FAB and the analysis is both more straightforward and faster.

Antibacterial Activity

Table 2 shows the *in vitro* antibacterial activity against *Bacillus subtilis* and 25 methicillin-resistant strains of clinical isolates of *Staphylococcus aureus* of all synthesized compounds in comparison with the parent Ere and vancomycin. The activity of compounds **Ib**, **IIa**, **IIb**, **IIId**, **Va** against *B. subtilis* is comparable with the activity of vancomycin and of eremomycin or is 2~4 times lower. Other compounds have lower antibacterial activity. Against *S. aureus*, **Ib**, **IIa**, **IIb**, **IIId** and **Va** are 4~8 times less active than eremomycin and comparable with vancomycin. Compounds **IIIe**, **IIIf** and **IVd** are moderately active; **IIIg**, **IVc**, **IVe**, **VIc**, **VId**, **VIe** have lower of activity.

Table 2. *In vitro* activity against *B. subtilis* and 25 MR strains of *S. aureus* of derivatives **I**~**VI** in comparison with eremomycin and vancomycin.

Compound	*B. subtilis* MIC (μg/ml)	*S. aureus* MIC_{50} (μg/ml)
Ere	0.16	0.5
Vancomycin	0.16	2
Ib	0.16	4
IIa	0.32	2
IIb	0.64	4
IIId	0.64	2
IIIe	1.28	8
IIIf	1.28	8
IIIg	3.20	64
IVc	1.92	32
IVd	1.28	8
IVe	1.60	16
Va	0.64	2
VIc	2.24	32
VId	1.60	16
VIe	1.92	32

Table 3. Antibacterial evaluation of compounds **Ib**, **IIa**, **IIb**, **IIId** and **Va** against series of Gram-positive bacteria in comparison with eremomycin and vancomycin.

Organism	MIC (μg/ml)						
	Eremo-mycin	Vanco-mycin	**Ib**	**IIa**	**IIb**	**IIId**	**Va**
Bacillus subtilis ATCC 6633	0.125	1	0.25	0.25	0.125	0.25	0.125
Corynebacterium xerosis NCTC 9755	0.25	1	≤0.06	0.125	0.125	0.125	≤0.06
Staphylococcus aureus Oxford	0.5	2	0.5	0.5	4	2	0.25
S. aureus Smith	0.25	2	0.5	1	4	1	0.5
S. aureus Russel	0.25	2	0.5	1	4	2	0.5
S. aureus V573	0.25	2	0.5	0.25	0.5	0.5	0.25
S. aureus V1100	2	2	0.5	4	16	2	0.5
S. aureus Wilson	0.5	2	0.5	2	1	2	1
S. epidermidis 54815	0.25	2	—	0.5	4	1	0.25
S. epidermidis NCTC11047	0.125	2	0.125	0.5	1	0.5	0.25
S, saprophyticus FL1	0.5	2	≤0.06	1	4	0.25	0.125
S. saprophyticus FL2	1	2	1	4	4	4	0.5
Micrococcus luteus NCTC8340	0.125	2	0.125	0.25	0.5	≤0.06	0.125
Streptococcus agalactiae 2798	0.125	1	≤0.06	≤0.06	0.125	≤0.06	≤0.06
S. agalactiae Hester	≤0.06	1	≤0.06	≤0.06	0.125	≤0.06	≤0.06
S. sanguis ATCC 10556	0.5	2	0.25	1	2	0.5	0.25
S. viridans Harding	≤0.06	1	≤0.06	≤0.06	0.125	≤0.06	≤0.06
S. faecalis I	0.5	2	0.5	1	2	0.25	0.5
S. faecalis T814	1	8	0.5	1	1	0.5	0.5

The most active compounds **Ib**, **IIa**, **IIb**, **IIId** and **Va** were investigated against a broad spectrum Gram-positive bacteria including clinical isolates (Table 3) in comparison with eremomycin and vancomycin. In this experiments, all glycopeptides were antibacterially active against Gram-positive bacteria. Antibacterial activity of **Va** and **Ib** was found to be comparable to that of Ere. Like Ere, the compounds **Ib**, **IIa**, **IIb**, **IIId**, **Va** were not significantly active against vancomycin-resistant enterococci[10] (*Enterococcus faecalis* NCTC 12201, *Enterococcus faecium* NCTC 12202, *E. faecalis* NCTC 12203. MIC > 64 μg/ml). *Escherichia coli* strains (*E. coli* ESS, *E. coli* NCTC 10418) were not sensitive to the Ere derivatives (MIC > 64 μg/ml).

Experimental

General Methods

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.

IR spectra were determined on a Pye-Unicam SP 1100 instrument in KBr, NMR spectra were recorded on a Varian VXR-400 spectrometer.

FAB mass spectra were obtained using a VG 2AB mass spectrometer fitted with an Ion Tech FAB gun. Samples were applied to the probe tip in a matrix of glycerol - thioglycerol - oxalic acid. Spectra were recorded in profile mode and several scans (10~15) were acquired into a single data file. The data was then smoothed, peak centroids determined and mass assigned using a series of data system procedures. Ionspray spectra were recorded on a Sciex API III mass spectrometer. Samples were flow injected into the mass spectrometer using 60% acetonitrile, 38% water, 2% acetic acid at a flow of 30 μl/minute.

Optical rotations were measured with a Perkin-Elmer-241 polarimeter. HPLC was performed on a DuPont 8800 liquid chromatograph, column (4.6 × 250 mm) Zorbax ODS 5 μm, detector SP8490 (λ 245 nm). Elution was carried out using linear gradient from 100% D to 100% F, where D: 5% CH_3CN - 95% 0.1 M $NH_4H_2PO_4$ and F: 45% CH_3CN - 55% 0.1 M $NH_4H_2PO_4$, in 25 minutes at the rate of 1 ml/minute. CM-cellulose column chromatography was performed on a LKB Ultrograd Gradient Mixer 11300, supplied with Uvicord 2138 and Recorder 2065. Preparative TLC was performed on the plates 20 × 20 cm coated with Merck Silica Gel $60F_{254}$ (1 mm). Pre-coated Merck Silica Gel $60F_{254}$ plates were used for TLC in systems: PrOH - EtOAc - conc. NH_4OH, 2:2:1 (A), 3:2:1 (B), 2:2:5 (C).

N,*N*-Dimethyleremomycin (**IIa**) and Methyl Ester of *N*,*N*-Dimethyleremomycin (**Va**)

$NaHCO_3$ (8 mg, 0.1 mmol) and CH_3I (0.04 ml 0.6 mmol) were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 12 ml of DMSO. The reaction mixture was stirred at room temperature for 6 hours. After addition of 200 ml of acetone the precipitated solid was collected by filtration and purified by CM-cellulose column chromatography (column 2 × 40 cm) on CM-32 (Whatman) during 8 hours using gradient of 0.2 M $AcONH_4$ (pH 6.7) - 0.2 M $AcONH_4$ with adding NH_4OH to pH 9.3. The solutions which contained **IIa** or **Va** were adjusted to pH 2 and passed through column (0.5 × 4 cm) of SDW-3 (H^+) resin elution being performed with 0.25 N NH_4OH. After concentration of the eluates to 3 ml and neutralization to pH 6 with 6 N H_2SO_4 100 ml of acetone was added to give the precipitates of **IIa** (148 mg, 45%) and **Va** (67 mg, 20%). **IIa**: Rf 0.31 (C), HPLC Rt 13.2 minutes. **Va**: Rf 0.48 (C), HPLC Rt 14.6 minutes.

N-Allyleremomycin (**Ib**) and *N*,*N*-Diallyleremomycin (**IIb**)

To a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 12 ml of DMSO, $NaHCO_3$ (33 mg, 0.4 mmol) and CH_2=$CHCH_2I$ (0.05 ml, 0.5 mmol) were added at room temperature. After 8 hours $NaHCO_3$ (33 mg) and CH_2=$CHCH_2I$ (0.05 ml) were added and 4 hours later the procedure was repeated. Two hundred ml of acetone were added to the reaction mixture. The precipitate was collected by filtration and purified by the method described for **IIa** and **Va** to yield 115 mg (35%) of **Ib** and 82 mg (25%) of **IIb**. **Ib**: Rf 0.64 (C), HPLC Rt 13.6 minutes. **IIb**: Rf 0.47 (C), HPLC Rt 15.2 minutes.

Benzyl Ester of *N*-Benzyleremomycin (**IVc**) and Benzyl Ester of *N,N'*-Dibenzyleremomycin (**VIc**)

$NaHCO_3$ (83 mg, 1 mmol) and $C_6H_5CH_2Cl$ (0.11 ml, 1 mmol) were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 13 ml of DMSO. The reaction mixture was stirred at room temperature for 20 hours and 0.11 ml of $C_6H_5CH_2Cl$ was added. Then the reaction mixture was stirred for 4 hours at 32°C and a product was precipitated with 150 ml Et_2O. It was collected by filtration and redissolved in 90 ml of a mixture of BuOH - H_2O (2 : 1). The solution was washed with H_2O (30 ml × 3), the organic layer was separated and evaporated. The purification was performd by preparative TLC (system B) to yield 70 mg (20%) of **IVc** and 44 mg (12%) of **VIc**. **IVc**: Rf 0.48 (A), 0.26 (B). **VIc**: Rf 0.57 (A), 0.40 (B).

Propyl Ester of Eremomycin (**IIId**), Propyl Ester of *N*-Propyleremomycin (**IVd**) and Propyl Ester of *N,N'*-Dipropyleremomycin (**VId**).

$NaHCO_3$ (50 mg, 0.6 mmol) and *n*-C_3H_7I (0.12 ml, 1.2 mmol) 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 23°C for 24 hours and a product was precipitated with 150 ml of Et_2O. The purification was performed by the method described for **IIa** and **Va** to yield 56 mg (17%) of **IIId**, 43 mg (13%) of **IVd** and 20 mg (6%) of **VId**. **IIId**: Rf 0.25 (A), 0.09 (B), HPLC Rt 16.6. **VId**: Rf 0.57 (A), 0.28 (B).

Heptyl Ester of Eremomycin (**IIIe**), Heptyl Ester of *N*-Heptyleremomycin (**IVe**) and Heptyl Ester of *N,N'*-Diheptyleremomycin (**VIe**).

(a) $NaHCO_3$ (50 mg, 0.6 mmol) and *n*-$C_7H_{15}I$ (0.1 ml, 0.6 mmol) were added to a stirred solution of eremomycin sulfate (330 mg, 0.2 mmol) in 12 ml DMSO. The reaction mixture was stirred at 22°C for 20 hours and a product was precipitated with 150 ml Et_2O. The purification was performed by the method described for **IVc** and **VIc** to yield 63 mg (18%) of **IIIe**, 60 mg (16%) of **IVe** and 47 mg (12%) of **VIe**. **IVe**: Rf 0.60 (A), 0.40 (B). **VIe**: Rf 0.65 (A), 0.46 (B).

(b) $NaHCO_3$ (33 mg, 0.4 mmol) and *n*-$C_7H_{15}I$ (0.08 ml, 0.5 mmol) were added to a stirred solution of eremomycin sulfate (165 mg, 0.1 mmol) in 7 ml DMSO. The reaction mixture was stirred at 40°C for 2 hours and a product was precipitated with 150 ml Et_2O and collected by filtration, redissolved in 90 ml of a mixture BuOH - H_2O (2 : 1). The resulting solution was washed with H_2O (50 ml × 3), 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 26 mg (15%) of **IIIe**. Rf 0.51 (A), 0.23 (B).

Dodecyl Ester of Eremomycin (**IIIf**)

$NaHCO_3$ (25 mg, 0.3 mmol) and *n*-$C_{12}H_{25}Br$ (0.1 ml, 0.4 mmol) were added to a stirred solution of eremomycin sulfate (165 mg, 0.1 mmol) in 8 ml DMSO. The reaction mixture was stirred at 40°C for 4 hours and a product was precipitated with 150 ml Et_2O. The purification was performed by the method described for **IIIe** (b) to yield 27 mg (15%) of **IIIf**. Rf 0.61 (A), 0.44 (B).

Octadecyl Ester of Eremomycin (**IIIg**)

$NaHCO_3$ (25 mg, 0.3 mmol) and *n*-$C_{18}H_{37}Br$ (50 mg, 0.15 mmol) were added to a stirred solution of eremomycin sulfate (165 mg, 0.1 mmol) in 8 ml DMSO. The reaction mixture was stirred at 40°C for 8 hours. The purification was performed by the method described for **IIIe** (b) to yield 25 mg (13%) of **IIIg**. Rf 0.61 (A), 0.54 (B).

Antibacterial Activities

Antibacterial activity *in vitro* against *Bacillus subtilis* ATCC 6633 was determined by the method of serial dilutions in tubes on broth; 1,000 ml of Hottinger hydrolysate with 28 mg % of amine N, 30 g of agar-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 method of agar serial dilutions. Agar plates containing 15 ml of agar containing two-fold dilutions of the test agents were inoculated to yield a final density of approximately 10^7 cfu per spot. The plates were incubated at 35°C for 24 hours in ambient air.

Minimal inhibitory concentrations (MIC's) of **Ib**, **IIa**, **IIb**, **IIId** and **Va** against an expanded spectrum Gram-positive bacteria (Table 3) were determined by the microtiter dilution method. 12 × 8 microtiter

trays had 50 μl broth per well. Todd-Hewitt broth was used for *Corynebacterium xerosis* NCTC 9755 and all enterococci and streptococci except *Streptococcus faecalis* I for which nutrient broth (Oxoid No 2) was used. Nutrient broth was used for all other cultures. Antibiotic solutions were in doubling dilutions. Inocula were 1 μl of a one tenth dilution of overnight broth cultures. Incubation was at 37°C overnight.

Acknowledgments

We gratefully acknowledge I. HOOD for fruitful discussion, M. GWYNN for antibacterial data (both from SmithKline Beecham Pharmaceuticals, Brockham Park, United Kingdom) and Dr. D. K. BRYANT (SmithKline Beecham Pharmaceuticals, Old Powder Mills, Tonbridge, Kent, United Kingdom) for ionspray mass spectrometry data.

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