EREMOMYCIN—NEW GLYCOPEPTIDE ANTIBIOTIC: CHEMICAL PROPERTIES AND STRUCTURE

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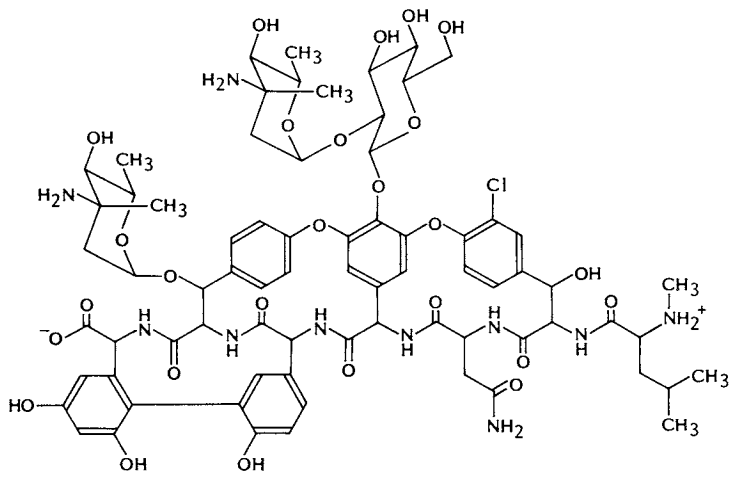
By a combination of chemical and spectroscopic (^1H and ^{13}C NMR) studies the structure of a glycopeptide antibiotic eremomycin has been elucidated. It is closely related to vancomycin, but differs in sugar and chlorine content. The eremomycin aglycone contains monodechlorovancomycinic acid; the only chlorine atom is situated in the second amino acid after the *N*-terminal amino acid residue of the peptide. The sugar part is composed of glucose and two residues of an amino sugar shown to be 2,3,6-trideoxy-3-amino-*C*-3-methyl-L-*arabino*-hexopyranose (4-*epi*-vancosamine). One of the amino sugar residues is a component of the disaccharide 2-*O*-(α-L-4-*epi*-vancosaminyl)-β-D-glucopyranose, attached to a triphenyl ether moiety; the position of another one is at the serine oxygen in the *C*-terminal region of the aglycone.

Eremomycin is identical to the recently obtained antibiotic A82846A and is very similar to orienticin A but differs from it only in the position of a chlorine.

Eremomycin was isolated from the culture filtrate of actinomycete numbered INA-238 by means of an ion exchange technique[1]. Taxonomic studies of the producing culture will be published elsewhere. Eremomycin was obtained as a homogenous crystalline sulfate and on the basis of its chemical and biological properties was identified as a new member of the vancomycin group of antibiotics[2].

Fig. 1. Structure of eremomycin.



† Deceased.

In vivo studies showed that eremomycin is more active and less toxic then vancomycin and ristomycin[3,4]. It does not cause local tissues damage and therefore may be used not only for intravenous but also for intramuscular injections. Eremomycin is now on clinical trial.

In this paper evidence for the structure of eremomycin is presented (Fig. 1).

Results and Discussion

The molecular formula of eremomycin $C_{73}H_{89}N_{10}O_{26}Cl$, MW 1,556 was determined by elemental analysis and fast atom bombardment (FAB)-MS, *m/z* 1,557.6 $(M+H)^+$. The physico-chemical properties of eremomycin sulfate ($C_{73}H_{89}N_{10}O_{26}Cl \cdot H_2SO_4$) are as follows: $[\alpha]_D^{20}$ $-100°$ (*c* 1, H_2O); isoionic point 8.3; UV $\lambda_{max}^{0.01\,N\,HCl}$ nm ($E_{1\,cm}^{1\%}$) 280 (42), $\lambda_{max}^{0.01\,N\,NaOH}$ 300 (60).

The bathochromic shift of maximum in UV spectrum indicated the presence of the phenolic groups. IR spectrum showed an absorption at 3350 cm^{-1} (NH/OH), intense amide peaks at 1650 and 1500 cm^{-1}; absorption at 1200~1000 cm^{-1} (C-O of sugars); a sharp band at 1220 cm^{-1} (C-O) typical of all known antibiotics of this group, apparently reflecting the presence of a triphenyl ether moiety.

Structure of the Aglycone

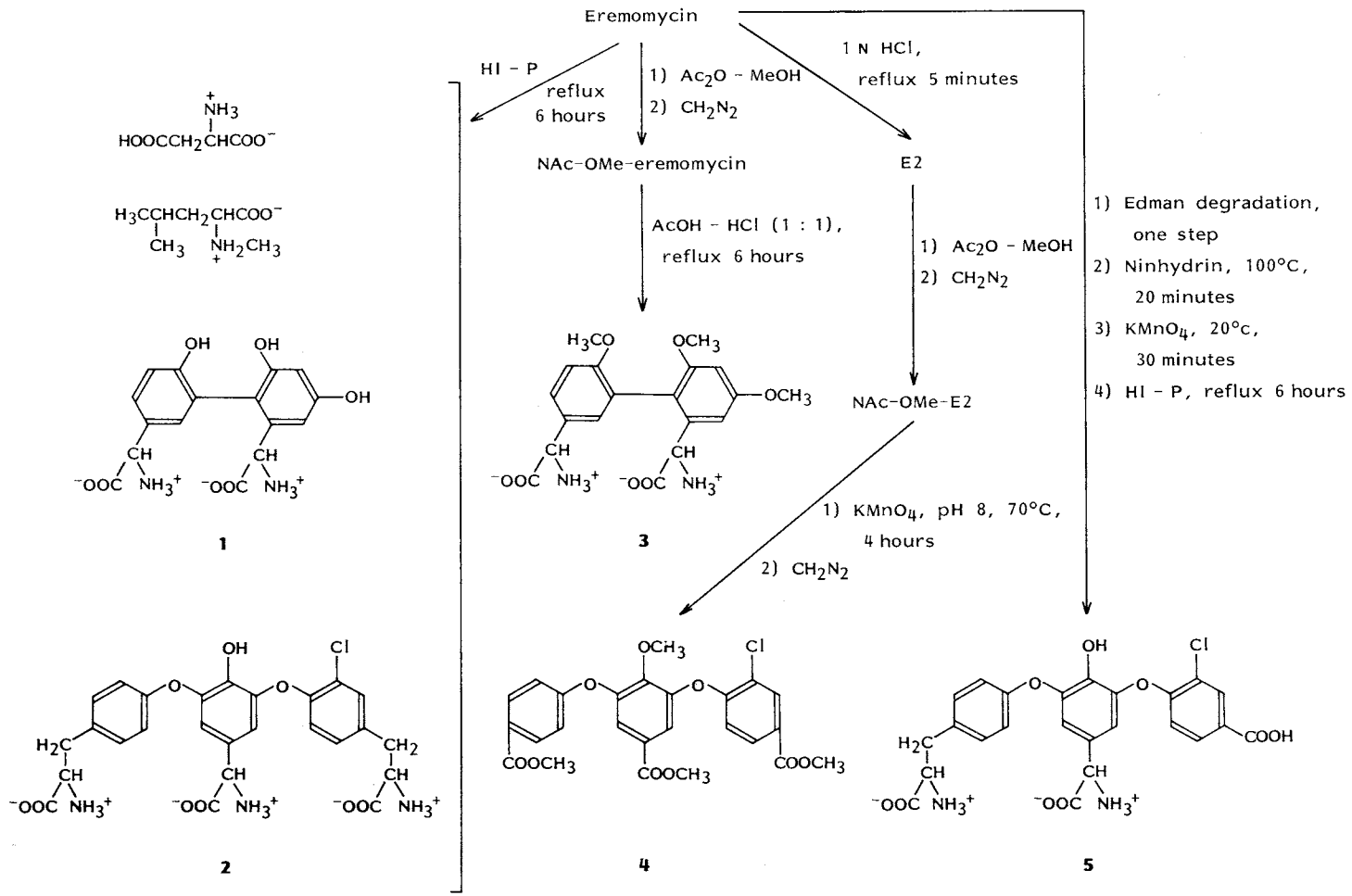
For the determination of amino acid content eremomycin was exposed to reductive hydrolysis with HI in presence of red phosphorous[5]. Four ninhydrin positive products were isolated from the hydrolysate and separated by ion-exchange column chromatography on Dowex 50X4 (Scheme 1). Two of them were identified as *N*-methyl-D-leucine and L-aspartic acid by paper chromatography, ^1H NMR spectroscopy and optical rotation. The structure of the biphenyl moiety **1** was confirmed by ^1H NMR spectrum of its *O*-methyl derivative **3**. Tri-amino-tri-carboxylic acid was found to be dideoxymonodechlorovancomycinic acid (**2**). ^1H NMR spectrum of this compound contained signals of nine aromatic protons and indicated the presence of one glycine and two alanine residues linked with benzene nuclei. The substitution patterns were confirmed by the ^1H NMR analysis of the oxidative degradation product **4**. Both **2** and **4** had only one chlorine atom.

Eremomycin contains the triphenoxy fragment with two residues of serine instead of alanine found in compound **2**. As will be shown later one of the alcoholic groups is responsible for the reaction of retroaldol cleavage and another one appeared to be glycosylated with an amino sugar.

To establish the structure of the peptide chain of eremomycin it was sufficient to determine the sequence of three amino acid residues at the *N*-terminal part of the molecule. For this purpose the antibiotic was subjected to Edman degradation. The first step resulted in parathyroid hormone(PTH)-*N*-methylleucine. On the second step, PTH-glycine was formed as a result of retroaldol destruction of the phenylserine fragment during the reaction with phenylisothiocyanate in alkaline solution. Therefore the serine residue with free hydroxyl is second in the sequence. On the third step of degradation with failed to distinguish PTH-aspartic acid. However free aspartic acid together with PTH-glycine were released on the second step of degradation of the peptide yielding partial hydrolysis of the aglycone but having the same amino acid content. This result demonstrated the cleavage of the peptide bond in the aglycone, involving the carboxyl of aspartic acid, and confirmed that this amino acid residue is the third after the *N*-terminal.

To clarify the position of chlorophenylserine residue eremomycin was exposed to one step of the Edman reaction and, after separation of *N*-methylleucine, was oxidized first with ninhydrin and then with

Scheme 1. Fragments of eremomycin chemical degradation.



potassium permanganate in neutral solution. An acidic compound isolated from HI-P hydrolysate was proved to be **5**. The ^{1}H NMR spectrum of **5** indicated the presence of glycine and, in contrast to **2**, only one alanine residue. The second one was replaced by a carboxyl group. This follows from the downfield shift of two aromatic proton signals; δ 7.84 (1H, dd, $J_{ortho}=8$ Hz, $J_{meta}=2$ Hz) and 8.00 (1H, d, $J_{meta}=2$ Hz). The other aromatic protons gave signals in the region 6.7~7.24 ppm. The splitting patterns of the downfield signals demonstrated that the carboxyl is situated on the chlorine-containing 1,2,4-substituted nucleus. This nucleus is the second after the *N*-terminal residue because that was the residue exposed to oxidation. It should be pointed out that all other antibiotics of the vancomycin group with monodechlorovancomycinic acid in their aglycones have a chlorine-containing residue in the region of the *C*-terminus. The structure of the eremomycin aglycone differs from that of vancomycin in the absence of one chlorine atom in the *C*-terminal part of molecule[6].

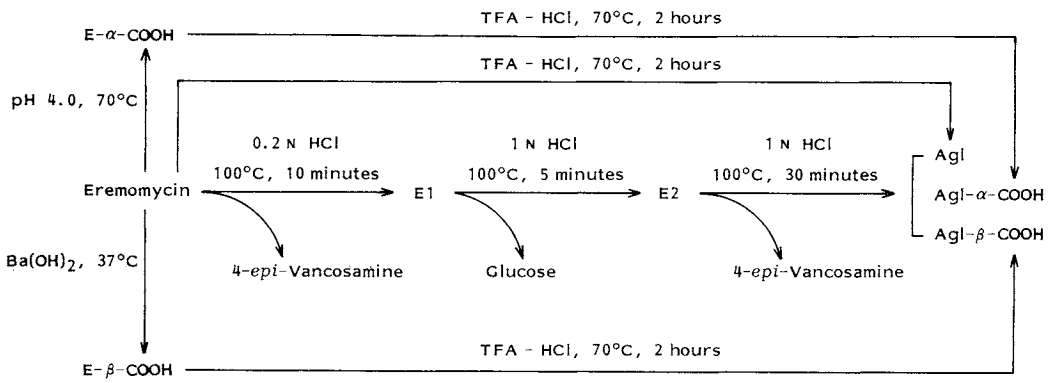
Structure and Position of Sugars

The carbohydrate moiety of eremomycin consists of one mol of glucose and two mol of an amino sugar, 4-*epi*-vancosamine. Mild acid hydrolysis of the antibiotic shows three stages of carbohydrate cleavage (Scheme 2). The identity of amino sugars isolated at the first and third stages was proved by comparing ^{1}H NMR spectra of their methyl *N,O*-acetylglycosides. Analysis of the spectral data suggested eremosamine to be 2,3,6-trideoxy-3-amino-*C*-3-methylhexose with axial protons at C-4 and C-5. It was designated as an L-sugar because the α-anomer of the derivative appeared to be levorotatory but the β-anomer was dextrorotatory. Equatorial orientation of the amine group at C-3 was established from the negative shift of the optical rotation value of methyl 4-*epi*-vancosaminide copper-ammonia complex[7].

On the basis of the above data the structure of 2,3,6-trideoxy-3-amino-*C*-3-methyl-L-*arabino*-hexopyranose (4-*epi*-vancosamine) was proposed for this amino sugar[8].

In order to determine the position of carbohydrate residues eremomycin and products of mild acid hydrolysis E1 and E2 were methylated with diazomethane to modify phenolic hydroxyls. Acid hydrolysates (AcOH-HCl, 1 : 1) of all derivatives were found to contain **3**. Therefore the phenolic groups of the biphenyl moiety are not substituted with sugars. Monodechlorovancomycinic acid was destroyed under HCl hydrolysis. To estimate its linkage with sugars the methylated eremomycin, E1 and E2 were oxidized with potassium permanganate in a weakly alkaline medium. A stable product of oxidation **4** obtained only from *O*-methyl E2, which had no glucose, demonstrated that the phenolic group of triphenyl ether moiety in eremomycin and E1 was substituted by this sugar.

Scheme 2. Sequence of carbohydrates liberation.



In E2 all phenolic groups are free, hence 4-*epi*-vancosamine can be linked in this compound only through one of the serine hydroxyls. It was already mentioned that the serine residue adjacent to the *N*-terminus has a free hydroxyl (release of PTH-glycine in process of Edman degradation). Thus the amino sugar in E2 is evidently located in the *C*-terminal part of the peptide.

The second molecule of 4-*epi*-vancosamine was shown to be attached to the aglycone through glucose. From the methanolysate of permethylated eremomycin (CH_3I, DMSO, powdered NaOH) methyl tri-*O*-methylglucoside was isolated whereas in the case of E1 under the same conditions methyl tetra-*O*-methylglucoside was obtained. Acetylation of the partly methylated methylglucoside and separation of the resulting products by silica gel column chromatography yielded α- and β-anomers of methyl tri-*O*-methyl-*O*-acetylglucoside. ^1H NMR spectra of these compounds revealed the presence of a lowfield signal at δ 4.7~4.8 near to the 1-H signal. It was assigned to 2-H because one of the coupling constants of this proton in the spectrum of the α-anomer was significantly less (4 Hz) than that in the spectrum of the β-anomer (8 Hz). Thus the disaccharide moiety of eremomycin is 2-*O*-(4-*epi*-vancosaminyl)-D-glucopyranose. The **1→2** type of glycoside bond is characteristic of the vancomycin group antibiotics.

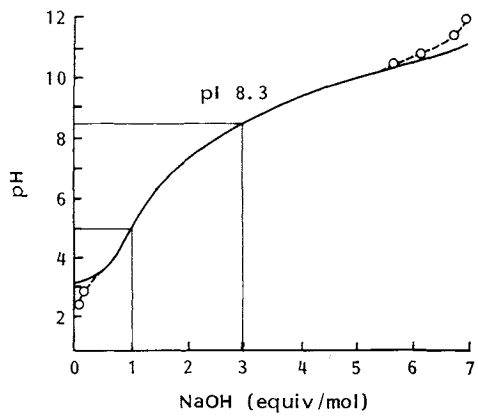
The configuration of the glycosidic links in eremomycin was determined by means of $^{13}C/^1H$ correlation NMR technique. The coupling constant $^1J_{CH}$ for the C-1 glucose residue was found to be 165 Hz indicating the β-confiugration. The constants 172.5 and 170.5 Hz for the C-1 amino sugar residues in the disaccharide moiety and attached through the serine hydroxyl (respectively) were in accordance with the α-configuration of their glycosidic bonds.

The Primary Amide Group and Related Reaction of Eremomycin

Eremomycin is an amphoteric substance. Potentiometric titration revealed three amino, one carboxyl and three phenolic groups in the molecule (Fig. 2). Isoionic point 8.3 was measured as a pH value of the solution deionized with suitable ion-exchangers.

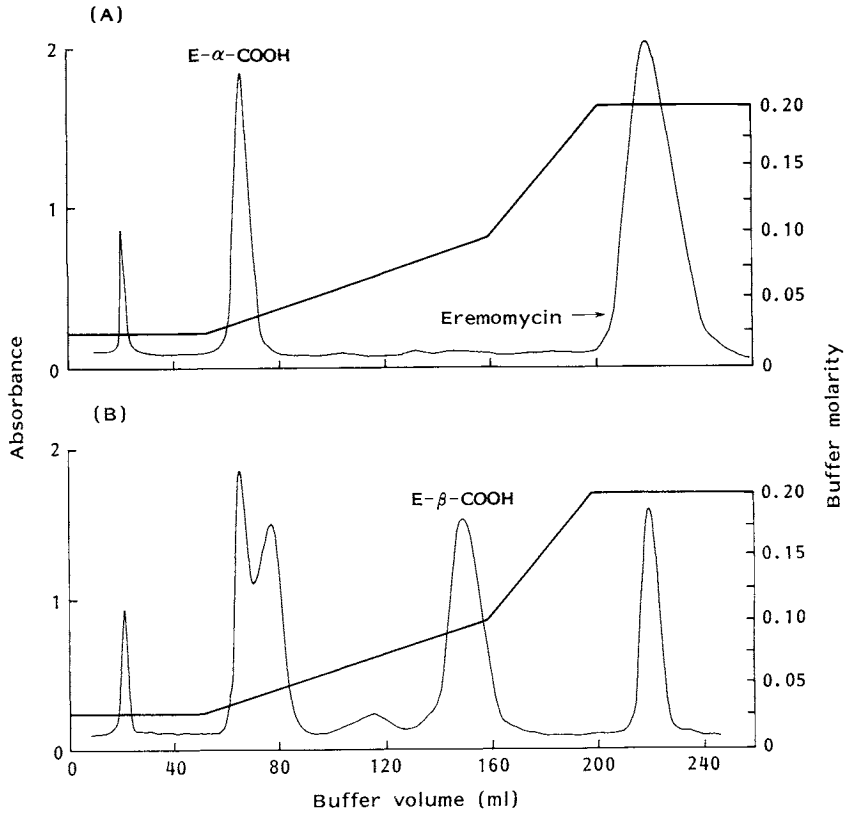
Eremomycin has no ester bonds (IR spectrum) but carries one primary amide that causes liberation of one mol ammonia at mild acid hydrolysis (1 N HCl, 105°, 1 hour). Its location on the asparagine residue follows from the ability of eremomycin to undergo α→β transpeptidation as described for vacomycin[9] and some peptides[10]. The rearrangement does not occur in the case of peptides containing aspartic acid residues with a free β-COOH.

Fig. 2. Acid-base titration curve of eremomycin (40 mg) in H_2O-EtOH, 8:2 (10 ml) with 0.25 N NaOH.
—— Experimental, ------ calculated.



Under conditions reported for vancomycin transformation (H_2O, pH 4.0, 70°C, 40 hours) eremomycin gave an inactive product (E-α-COOH) with a net charge of +1 (three amino and two carboxyl groups) which was separated by CM-cellulose column chromatography in a gradient of ammonium acetate (Fig. 3). Another compound with the same charge but possessing significant activity was isolated from the solution of antibiotic in saturated $Ba(OH)_2$ after 3~5 hours incubation at 37°C. It differed from E-α-COOH in chromatographic behavior (CM-cellulose column, TLC, PC)

Fig. 3. Column chromatography.



(A) Eremomycin heated at pH 4.0, 70°C, 40 hours, (B) eremomycin exposed in satd soln $Ba(OH)_2$ at 37°C, 3 hours (column: CM-cellulose, H = 36 cm, eluent: 0.025 ~ 0.2 M ammonium acetate gradient).

and was assumed to be deamidoeremomycin (E-β-COOH).

Deglycosylation of eremomycin during mild acid hydrolysis was also followed by the reactions of transpeptidation and deamidation. After all carbohydrates had been released (1 N HCl, 100°C, 1 hour) three aglycones were found in the hydrolysate; a neutral (trace amounts) and two acidic aglycones with an overall charge of −1 (one amino and two carboxyl groups). They were identified as Agl-$CONH_2$, Agl-β-COOH and Agl-α-COOH on the basis of their relation of eremomycin, E-β-COOH and E-α-COOH (respectively) established by the method of specific deglycosylation with anhydrous TFA-HCl[11]. Only a neutral aglycone was obtained from eremomycin indicating that deamidation and transpeptidation do not take place under these conditions. In contrast to the other aglycones, Agl-α-COOH was composed of two closely related components (TLC, EtOAc-BuOH-AcOH-H_2O, 7:2:4:2), supposed to be geometric isomers, differing in the orientation of chlorine atom.

When this manuscript was in preparation, the structures of the glycopeptide antibiotics orienticins[12] and A82846[13] were reported. Among them the antibiotic A82846A was found to be identical to eremomycin and orienticin A differs from it only in containing a chlorine in the phenylserine residue, the second after the *C*-terminus instead of in the second after the *N*-terminus.

Experimental

General

UV spectra were determined on a Beckman UV 5260 spectrometer, IR spectra were determined on a Beckman 620 MX instrument in KBr, NMR spectra were recorded on a Varian XL-100 and Bruker WP-200 SY spectrometers. Chemical shifts (δ) were expressed in ppm downfield from an internal TMS reference. A molecular weight was obtained by FAB-MS on a Kratos MS-50 instrument. Optical rotations were measured with a Perkin-Elmer polarimeter. Potentiometric titration was performed on a titrator TTT-1 supplied with titrigraph SBR-2 and microburette SBU-1 (Radiometer, Denmark), with glass and calomel electrodes in solution H_2O-EtOH, 4:1. The data below pH 3 and above pH 10 were corrected by means of blank titration. CM-cellulose column chromatography was performed on a LKB Ultrograd gradient mixer 11300 with Uvicord 2138 and Recorder 2065. Precoated Merck Silica gel 60 F_{254} plates were used for TLC in systems PrOH-EtOAc-concd NH_4OH, 1.5:1.5:2.0 (E, E1, E2, Agl *et al.*); $CHCl_3$ (PTH amino acids); $CHCl_3$-MeOH, 9:1 and C_6H_6-Me_2CO, 7:3 (sugar derivatives). Whatman N2 was used for paper chromatography in systems BuOH-AcOH-H_2O, 2:1:1 (amino acids); BuOH-C_5H_5N-AcOH-H_2O, 15:10:3:12 (amino acids, amino sugars); BuOH-C_5H_5N-PrOH-AcOH-H_2O, 20:10:5:3:32, upper phase (E, E1, E2, Agl, E-β-COOH, E-α-COOH, Agl-β-COOH, Agl-α-COOH). Paper electrophoresis was carried out in 2 N AcOH at 700 V for 3 hours and in 0.5 M C_5H_5N-AcOH buffer pH 5.6 at 900 V for 3 hours.

Acid Hydrolysis of Eremomycin

A solution of eremomycin (100 mg/ml) was heated in 57% HI with red phosphorus (20 mg/ml) for 6 hours or in 2 N HCl for 12 hours under reflux. Amino acids were separated by column chromatography on Dowex 50X4 using 0.1 N pyridine-AcOH buffer pH 3.65 for aliphatic and pH 4.7 for aromatic amino acids.

L-Aspartic acid crystallized from H_2O-EtOH, 3:1: $[\alpha]_D^{20}$ +23.5° (*c* 5, 5 N HCl); ^{1}H NMR (100 MHz, DMSO+TFA) δ 3.44 (2H, d, J=6 Hz, CH_2), 4.68 (1H, t, α-CH).

N-Methyl-D-leucine crystallized from H_2O-EtOH, 1:1: $[\alpha]_D^{20}$ −19.2° (*c* 5, 5 N HCl); ^{1}H NMR (100 MHz, DMSO+TFA) δ 0.95 (6H, d, J=6 Hz, 2×CH_3), 1.7 (3H, m, β-CH_2 and γ-CH), 2.68 (3H, s, $NHCH_3$), 3.65 (1H, t, α-CH).

Dideoxymonodechlorovancomycinic acid (**2**): *Anal* calcd for $C_{26}H_{26}N_3O_9Cl$: Cl 6.26; found: Cl 6.02; ^{1}H NMR (100 MHz, DMSO+TFA) δ 3.1 (4H, d, J=6 Hz, 2×CH_2), 4.18 (2H, m, 2×α-CH), 4.98 (1H, br s, α-CH), 6.7~7.5 (9H, aromatic).

N-Acetylation and Methylation

N-Acetylation and methylation of eremomycin, E1 and E2 were carried out by the method described previously[14].

Tri-*O*-methylactinoidinic Acid (3)

A solution of *N*-acetyl-*O*-methyleremomycin in AcOH-10 N HCl, 1:1 (100 mg/ml) was refluxed for 6 hours. After filtration and evaporation of the hydrolysate the residue was dissolved in water and the solution adjusted to pH 7.0 was applied to a column (H=50 cm) of Sephadex G-25 developed with 0.1 N AcOH in H_2O-EtOH, 9:1. The fractions were analyzed by paper chromatography. Compound **3** was isolated as a white foam: ^{1}H NMR (100 MHz, CD_3OD) δ 3.62 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.54 (1H, s, α-CH), 4.97 (1H, s, α-CH), 6.63 (2H, d, J=2.5 Hz), 7.07 (1H, d, J=8 Hz), 7.32 (1H, d, J=2 Hz), 7.46 (1H, dd, J_1=2 Hz, J_2=8 Hz).

Compound 4

Compound **4** was prepared by the oxidation of *N*-acetyl-*O*-methyl derivative of E2 with $KMnO_4$ by the previously reported method[15]. After filtration the solution was acidified (pH 2.0) and the insoluble fraction was separated. *Anal* calcd for $C_{21}H_{13}O_9Cl$: Cl 7.88; found: Cl 7.30. After esterification with diazomethane the resulting product was purified by Kieselgel 60 (Merck) column chromatography using C_6H_6 with increasing amounts of $CHCl_3$ to give **4**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 3.74 (3H, s, OCH_3),

3.82 (3H, s, OCH_3), 3.88 (6H, s, $2\times OCH_3$), 6.79 (1H, d, $J=8$ Hz), 6.9 (2H, d, $J=8$ Hz), 7.48 (1H, d, $J=2$ Hz), 7.56 (1H, d, $J=2$ Hz), 7.83 (1H, dd, $J_1=2$ Hz, $J_2=8$ Hz), 7.97 (2H, d, $J=8$ Hz), 8.08 (1H, d, $J=2$ Hz).

Compound **5**

Compound **5** was obtained from eremomycin by the method previously described for the preparation of "amino acid Z" from actinoidin[16]. The purification of **5** was carried out on Sephadex G-25 column (H=80 cm) with eluent 0.1 N AcOH in H_2O-EtOH, 9:1. After concentration of the fraction **5** was precipitated with acetone. R_{glu} 0.28 (electrophoresis, buffer pH 5.6): ^{1}H NMR (100 MHz, DMSO+TFA) δ 3.1 (2H, d, $J=6$ Hz, CH_2), 4.08 (1H, br s, α-CH), 4.95 (1H, br s, α-CH), 6.76~7.02 (5H, m, aromatic), 7.24 (2H, d, $J=8$ Hz), 7.84 (1H, dd, $J_1=2$ Hz, $J_2=8$ Hz), 8.0 (1H, d, $J=2$ Hz).

Edman Degradation

The ethyl acetate extract after the first step of degradation was evaporated and purified by Kieselgel 40 (Merck) column chromatography (C_6H_6 with increasing amounts of $CHCl_3$) to give PTH-*N*-methylleucine: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.0 (6H, d, $J=6$ Hz, $2\times CH_3$), 1.92 (3H, m, β-CH, γ-CH), 3.34 (3H, s, $NHCH_3$), 4.12 (1H, t, α-CH), 7.16~7.50 (5H, m, aromatic).

The aqueous solution after extraction was adjusted to pH 3.0 with Dowex 2X4 $(OH)^-$ and evaporated, the residue was exposed to the second step of degradation. PTH-glycine was identified by direct comparison with an authentic sample.

4-*epi*-Vancosamine

A solution of eremomycin sulfate (7 g) in 1 N HCl (70 ml) was refluxed for 1 hour, then cooled, filtered, diluted with H_2O (1:4) and poured out through the column (20 g) of SDW-3 $(H)^+$ (analog of Dowex 50X2, 100 mesh). The resulting solution was adjusted to pH 6 with anion exchanger and passed through column (10 g) of SDW-3 $(H)^+$. Elution was performed with 0.5 N HCl. After neutralization of the eluate with anion exchanger (pH 6.0) solvent was evaporated and the residue treated with EtOH to separate mineral salts. The removal of EtOH yielded 0.9 g of 4-*epi*-vancosamine as a white foam: $[\alpha]_D^{20}$ $-19.3°$ (*c* 1.56, MeOH).

Hydrolysis of E2 (3.5 g) under the same condition gave 0.25 g of 4-*epi*-vancosamine.

Methyl 4-*epi*-Vancosaminide

A solution of 4-*epi*-vancosamine (0.38 g) in 1 N HCl methanolic solution (38 ml) was heated for 1 hour under reflux. The methanolysate after cooling was diluted with ice-water, neutralized with anion exchanger (pH 6.0) and evaporated. The resulting residue was crystallized from MeOH-Et_2O, 1:5 to yield 50 mg of α-methyl-4-*epi*-vancosaminide: $[\alpha]_D^{20}$ $-116°$ (*c* 1.74, MeOH); ^{1}H NMR (100 MHz, CD_3OD) δ 1.28 (3H, d, $J=6$ Hz, CH_3), 1.48 (3H, s, CH_3), 2.08 (2H, br s, CH_2), 3.30 (3H, s, OCH_3), 3.37 (1H, d, $J=10$ Hz, 4-H), 3.67 (1H, dq, $J_1=6$ Hz, $J_2=10$ Hz, 5-H), 4.7 (1H, dd, $J_1=3$ Hz, $J_2=2$ Hz, 1-H).

Methyl *N*,*O*-Acetyl-4-*epi*-vancosaminide

The solution resulted after separation of α-methyl-4-*epi*-vancosaminide (see above) was evaporated the residue dissolved in C_5H_5N (2 ml), acetic anhydride (1.2 ml) was added and mixture was left overnight at room temperature, then poured into H_2O (20 ml) and extracted with $CHCl_3$ (20 ml × 3). The combined extract was washed with 0.5 N H_2SO_4 and H_2O. After removal of $CHCl_3$ the residue was purified by Kieselgel 40 Merck column chromatography developed with C_6H_6-$(CH_3)_2CO$, 9:1. Two main products were afforded as white crystals:

Methyl *N*,*O*-Acetyl-α-L-4-*epi*-vancosaminide: $[\alpha]_D^{20}$ $-81.7°$ (*c* 1.96, MeOH); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.14 (3H, d, $J=6$ Hz, CH_3), 1.52 (3H, s, OCH_3), 1.82 (3H, s, $NHCOCH_3$), 2.10 (3H, s, $CCOCH_3$), 2.20 (1H, dd, $J_1=14$ Hz, $J_2=5$ Hz, CH_2 ax), 2.63 (1H, d, $J=14$ Hz, CH_2 eq), 3.28 (3H, s, OCH_3), 3.83 (1H, dq, $J_1=6$ Hz, $J_2=10$ Hz, 5-H), 4.64 (1H, d, $J=5$ Hz, 1-H), 4.77 (1H, d, $J=10$ Hz, 4-H), 6.08 (1H, s, NH).

Methyl *N*,*O*-Acetyl-β-L-4-*epi*-vancosaminide: $[\alpha]_D^{20}$ $+42.5°$ (*c* 1.19, MeOH); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.23 (3H, d, $J=6$ Hz, CH_3), 1.52 (3H, s, CH_3), 1.76 (1H, m, CH_2 ax), 1.87 (3H, s, $NHCOCH_3$),

2.14 (3H, s, $OCOCH_3$), 2.86 (1H, dd, $J_1=14$ Hz, $J_2=2.5$ Hz, CH_2 eq), 3.46 (3H, s, OCH_3), 3.67 (1H, dq, $J_1=6$ Hz, $J_2=10$ Hz, 5-H), 4.46, (1H, dd, $J_1=10$ Hz, $J_2=2.5$ Hz, 1-H), 4.62 (1H, d, $J=10$ Hz, 4-H), 6.32 (1H, s, NH).

Methyl 4-*epi*-Vancosaminide Cuprammonium Complex

The solution (3 ml) of methyl eremosaminide in water was evaporated, the residue was dissolved in 3 ml 4% tetrammincopper(11) sulfate (TACu). The optical rotation was measured at 436 nm: $[\alpha]_{H_2O}$ $-0.192°$, $[\alpha]_{TACu}$ $-0.856°$.

Methyl 2-*O*-Acetyl-3,4,6-tri-*O*-methyl-D-glucopyranoside, α- and β-Anomers

A mixture of eremomycin (1.5 g), DMSO (24 ml), powdered NaOH (3 g) and CH_3I (3.7 ml) was stirred at room temperature for 2 hours. Then NaOH was filtered off and the solution extracted with $CHCl_3$. After washing with water the extract was evaporated and the residue reprecipitated with ether from EtOH. The resulting product was dissolved in 1 N HCl - MeOH and the solution (40 mg/ml) was heated under reflux for 2 hours then cooled and diluted with H_2O (1 : 1). The precipitate was filtered off and the solution was adjusted to pH 6.5 with anion-exchanger and evaporated. The residue was treated with ethanol - ether, 1 : 10. The soluble fraction was purified by column chromatography (Kieselgel 40 Merck, 1×27 cm, C_6H_6 - Me_2CO, 9 : 1) and acetylated with acetic anhydride in pyridine (1 : 1.5) at 20°C for 18 hours. After removal of the solvent the residue, applied to Kieselgel 40 Merck column (1×25 cm), yielded two main products, as a syrupy substances.

Methyl 2-*O*-Acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranoside (eluted C_6H_6 - $CHCl_3$, 7 : 3): ^1H NMR (100 MHz, $CDCl_3$) δ 2.12 (3H, s, $OCOCH_3$), 3.37 (3H, s, OCH_3), 3.41 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 4.7 (1H, dd, $J_1=4$ Hz, $J_2=9.5$ Hz, 2-H_{ax}), 4.83 (1H, d, $J=4$ Hz, 1-H_{eq}).

Methyl 2-*O*-Acetyl-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (eluted C_6H_6 - $CHCl_3$, 1 : 1): ^1H NMR (100 MHz, $CDCl_3$) δ 2.07 (3H, s, $OCOCH_3$), 3.38 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.48 (3H, s, OCH_3), 3.5 (3H, s, OCH_3), 4.2 (1H, d, $J=8$ Hz, 1-H_{ax}), 4.8 (1H, dd, $J_1=8$ Hz, $J_2=8$ Hz, 2-H_{ax}).

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