2-EPIMUTALOMYCIN AND 28-EPIMUTALOMYCIN, TWO NEW POLYETHER ANTIBIOTICS FROM *STREPTOMYCES MUTABILIS*

DERIVATIZATION OF MUTALOMYCIN AND THE STRUCTURE ELUCIDATION OF TWO MINOR METABOLITES

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A number of derivatives of mutalomycin (**1**), a naturally occurring polyether antibiotic, have been synthesized. In the desulfurization reaction of the ethylthio derivative (**5**) of mutalomycin (**1**) with Raney-nickel we observed an unusual course of the reaction, namely the introduction of a hydroxy group instead of the usual exchange against hydrogen, leading to two reaction products, mutalomycin (**1**) and 28-epimutalomycin (**3**). The structure of **3** and 2-epimutalomycin (**2**), both minor metabolites from the mutalomycin fermentation, were elucidated by X-ray analysis.

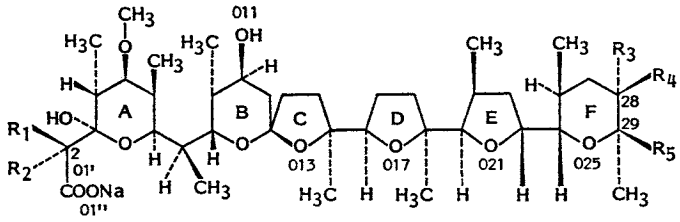
In the course of our search for new antibiotics from actinomycetes, we found a new polyether antibiotic, which we named mutalomycin (**1**). The structure was elucidated by X-ray analysis[1,2] and the assignments of the ^{13}C NMR spectra were made[3]. We also isolated two new minor metabolites, their structures are not published yet. By now, more than 100 naturally occurring polyethers are known[4]. They are not only powerful antibiotics, they also exhibit strong positive inotropic effects on the heart muscle[5]. Unfortunately most of these compounds are very toxic and therefore only of limited interest for human applications. With the aim to reduce toxicity we prepared a number of derivatives of mutalomycin (**1**).

Results and Discussion

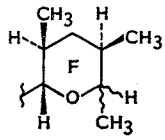
The sodium salt of mutalomycin (**1**) is a stable compound and can be kept in methanol or other solvents without any changes. The free acid on the other hand reacts with methanol forming a methyl ether derivative (**4**). This reaction can be catalyzed by addition of an acid, *e.g.* acetic acid. In the same manner the ethyl thioether derivative of mutalomycin (**5**) is formed with ethanethiol in the presence of acetic acid. From the elemental analysis and NMR spectral data it was estimated that in the molecule of mutalomycin one hydroxy group was exchanged against a methoxy and an ethylthio-function respectively.

Because of conformational instability in solution a full interpretation of the ^{1}H and ^{13}C NMR spectra of the derivatives was not possible and therefore we could not locate at which C-atom the substitution had occurred. As it was shown on the structurally related polyether antibiotics A28695B[6] and maduramicin[7] under normal conditions the hemiketal function of ring F reacts with alcohols forming ketals. In analogy to this we assumed that in mutalomycin also the hemiketal function of ring

Fig. 1. Structural formulas.



Mutalomycin (**1**)	$R_1=CH_3$	$R_2=H$	$R_3=H$	$R_4=CH_3$	$R_5=OH$
2-Epimutalomycin (**2**)	$R_1=H$	$R_2=CH_3$	$R_3=H$	$R_4=CH_3$	$R_5=OH$
28-Epimutalomycin (**3**)	$R_1=CH_3$	$R_2=H$	$R_3=CH_3$	$R_4=H$	$R_5=OH$
29-Deoxy-29-methoxymutalomycin (**4**)	$R_1=CH_3$	$R_2=H$	$R_3=H$	$R_4=CH_3$	$R_5=OCH_3$
29-Deoxy-29-ethylthiomutalomycin (**5**)	$R_1=CH_3$	$R_2=H$	$R_3=H$	$R_4=CH_3$	$R_5=SC_2H_5$



29-Deoxymutalomycin (**6**)
$R_1=CH_3$ $R_2=H$ $R_3=H$ $R_4=CH_3$

F has undergone ketalization. Field desorption mass spectroscopy (FD-MS) is quite useful for the structure determination of polyether ketals as was shown for A28695B[6]. Examination of the FD-MS of our etheral derivatives and especially their methyl esters revealed the significant fragment peaks for a structure in which ketalization has occurred in ring F.

This proves the structures **4** and **5** for 29-deoxy-29-methoxymutalomycin and 29-deoxy-29-ethylthiomutalomycin respectively leaving open the stereochemistry at C-29. Careful NMR and X-ray studies on ketals of maduramicin, a polyether antibiotic with the same substitution pattern on ring F, showed that the ketalization occurred on ring F with retention of the stereochemistry at C-29[7]. In analogy to the stereochemical course of the ketalization on maduramicin we propose that **4** and **5** have the stereochemistry as indicated in Fig. 1.

In an other attempt we tried to prepare the 29-deoxy derivative of mutalomycin (**6**) by the desulfurization of 29-deoxy-29-ethylthiomutalomycin (**5**) with Raney-nickel. In the reaction mixture, two products were detected in the ratio 2:3.

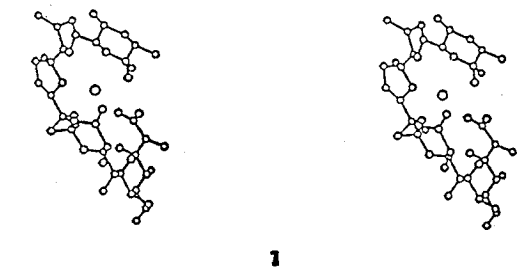
Chromatographic separation of the two components showed that the first eluting compound was identical with mutalomycin (**1**) and the second one (**3**) had the same molecular formula as **1** according to the elemental analysis and the mass spectrum. Component **3** proved to be identical with the less polar metabolite of two minor ones (**2** and **3**) which we isolated from a large scale fermentation of mutalomycin.

From these results it can be deduced that **1** and **3** are isomers. Furthermore the desulfurization reaction did not work in the expected way because the thioethyl group was exchanged against a hydroxy function instead of a hydrogen. This unusual behavior was observed only in hemithioketals where the oxygen and the sulfur atoms are in a 1, 3 position of the same 5 or 6 membered ring. The hemithioacetal desulfurization drawn from carbohydrate chemistry always replaces the sulfur by hydrogen without attacking the carbon oxygen bond[8].

The synthesis of the deoxy derivative (**6**) could finally be achieved by the hydrogenation of **1** with palladium on charcoal as catalyst. The sodium salt of the reaction product showed a molecular peak in the FD-MS which was 16 mass units smaller than for mutalomycin (**1**). The fragmentation pattern of the methyl ester of **6** showed the expected mass peaks for the ring fragments DEF, EF and F and the NMR spectra were in accordance with the proposed structure **6** without proof of the stereochemistry at C-29.

The structures of the already mentioned new metabolites (**2** and **3**) could not be elucidated by NMR spectroscopy alone because of the conformational instability in different organic solvents used. But from elemental analysis and the FD-MS it was deduced that **2** and **3** possess the same molecular formula as mutalomycin (**1**) and therefore are isomers. Since **3** can also be prepared from mutalomycin (**1**) *via* the desulfurization of the thioethylketal (**5**) one would expect **3** to be the 29-epimer of mutalomycin; the entering hydroxy group could attack C-29 from both sides. But the X-ray analysis of the potassium salt of **3** showed that it is the 28-epimer. This unexpected result was also observed with epinigericin[9] a new polyether carboxylic antibiotic isolated from *Streptomyces hygroscopicus* whose structure was investigated by 1D and 2D ^{1}H and ^{13}C NMR spectrometry. Epinigericin was converted to nigericin in low yield by acid catalysis. The epimerization at C-28 of epinigericin was explained by the opening of the ring F to the ketonic intermediate, which is isomerized at C-28 to the more stable ketone, which cyclizes again to nigericin. The result of the X-ray analysis of the potassium salt of the minor metabolite (**2**) showed that it is 2-epimutalomycin

Fig. 2. Stereo drawings of the X-ray crystal structures of mutalomycin (**1**), 2-epimutalomycin (**2**) and 28-epimutalomycin (**3**).



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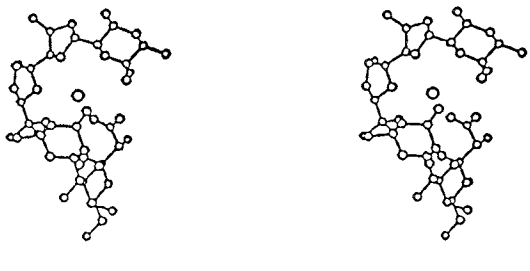

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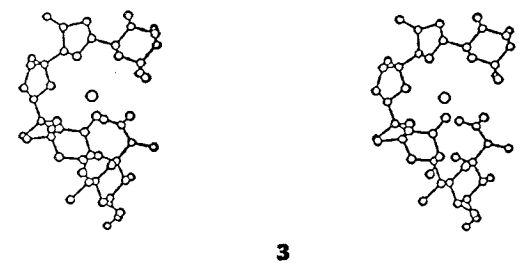

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Table 1. Coordination of K^+ and intramolecular hydrogen bonding distances (Å) in the crystal structures of mutalomycin (**1**), 2-epimutalomycin (**2**) and 28-epimutalomycin (**3**).

	1	2	3
K···O1′	2.702	2.666	2.679
K···O1″	3.250	3.079	2.789
K···O11	2.698	2.651	2.665
K···O13	2.666	2.714	2.745
K···O17	2.842	2.846	2.910
K···O21	2.761	2.697	2.701
K···O25	2.982	3.051	3.084
K···O29	2.972	3.201	3.477
O1′··O3	2.656	2.661	2.607
O1″·O29	2.623	2.661	2.965

Table 2. Toxicity and cardiovascular activity (LD_{50} values on mice).

	1	2	3	4	5	6
ip (mg/kg)	8	—	20	70	>20	20
iv (mg/kg)	3	—	<10	32	>10	>10

Mutalomycin (**1**) was tested on the 'open-chest' dog with numal as narcoticum. It showed a positive inotropic effect similar to digitalis, but on contrary to that, a strong dilatation of the coronary vessels and an increase of the oxygen saturation of the coronary veins was observed.

28-Epimutalomycin (**3**) showed activity very similar to mutalomycin (**1**) and 29-deoxy-29-ethylthio-mutalomycin (**5**) was inactive.

2-Epimutalomycin (**2**) was not tested at all.

(**2**). Also in this molecule the epimerization occured on the α-carbon atom to the hemiketal function and not at the hemiketal function itself.

The potassium salts of **1**~**3** take similar conformations in the crystal (Fig. 2). In all three cases the molecules manage to adopt a conformation such that the carboxyl group, the hydroxyl group on ring B and the ether oxygen atoms from rings C~F all coordinate to the potassium ion (Table 1). The circular conformation of the polyether is further clipped in place by an intramolecular hydrogen bond between a carboxy oxygen atom and the hydroxyl group (O-29) on ring F. The other striking conformational feature is a strong intramolecular hydrogen bond between the carboxyl group and O-3 (the hydroxyl group on ring A) which locks the conformation of the carboxy chain.

2-Epimutalomycin (**2**) crystallizes with two water molecules in the lattice which form hydrogen bonds to O-1′, O-1″ and O-11, but do not coordinate to the K^+ ion.

Experimental

All mp's were determined on a Tottoli melting point apparatus and are uncorrected. The mass spectra were recorded on a CEC 21-110B or MAT 212 spectrometer (electron ionization and field desorption spectra). The IR spectra were obtained on a Perkin-Elmer spectrometer model 21 with filter and the UV spectra on a Beckmann photometer model DK 2 in methanol. The ^1H NMR spectra in $CDCl_3$ were determined on a Bruker WH-360 spectrometer; internal standard, TMS 0 ppm.

TLC was performed on precoated Silica gel 60 F_{254} plates (Merck) with toluene - acetone - triethylamine (80 : 20 : 1).

The methyl esters were prepared in the usual way by reacting the free acid forms with diazomethane.

Preparation of 29-Deoxy-29-methoxymutalomycin (**4**)

Mutalomycin sodium salt (5 g, 6.4 mmol) in CH_2Cl_2 (250 ml) at 0°C were extracted twice with 1 N HCl (250 ml) and H_2O (250 ml), dried with Na_2SO_4 and evaporated to dryness. The residue, the free acid (4.82 g), was kept in MeOH (50 ml) for 15 hours at 25°C. MeOH was evaporated, the residue diluted with CH_2Cl_2 (100 ml) and washed twice with 1 N NaOH (100 ml) and H_2O (100 ml). After drying and evaporating of the solvent, the sodium salt of **4** was purified by chromatography on silica gel (Merck 05) (300 g). Crystallization and recrystallization of one of the pure fractions from MeOH gave colorless crystals: MP 150~154°C (dec); $[\alpha]_D^{22}$ +102° (*c* 1.0, $CHCl_3$); IR ν_{max} (CH_2Cl_2) cm^{-1} 2990, 2900, 2890, 1595, 1470, 1390, 1240, 1100, 1075, 1040, 1020, 970, 940, 870; ^1H NMR δ 3.18 (3H, s, OCH_3), 3.34 (3H, s, OCH_3); FD-MS *m/z* 791 (MH^+, $C_{42}H_{71}O_{12}Na$), 529, 444, 240 (EF, $C_{14}H_{24}O_3$), 158 (F, $C_9H_{18}O_2$).

Anal Calcd for $C_{42}H_{71}O_{12}Na$ (791.01): C 63.8, H 9.0, O 24.3, Na 2.9.
Found: C 63.9, H 9.1, O 24.0, Na 3.2.

Methyl Ester: FD-MS *m/z* 783 (MH^+, $C_{43}H_{74}O_{12}$), 541 (ABCD, $C_{29}H_{49}O_9$), 457 (ABC, $C_{24}H_{41}O_8$), 325 (DEF, $C_{19}H_{33}O_4$), 241 (EF, $C_{14}H_{25}O_3$), 158 (F, $C_9H_{18}O_2$).

Preparation of 29-Deoxy-29-ethylthiomutalomycin (**5**)

Mutalomycin (1 g free acid, 1.32 mmol) was treated with ethanethiol (5 ml, 67.5 mmol) and acetic acid (2 ml) at room temperature for 5 days until TLC showed complete conversion of the educt ($CHCl_3$ - MeOH, 95 : 5). The reaction mixture was neutralized with a solution of NH_3 in ethanol and evaporated to dryness. The residue was chromatographed on Silica gel G (Merck, 180 g). Elution with $CHCl_3$ - MeOH (97 : 3) afforded **5** as a white foam. Crystallization of 400 mg of this portion from hexane yielded white crystals (211 mg) of the free acid: MP 142~148°C (dec); $[\alpha]_D^{22}$ +104° (*c* 1.1, $CHCl_3$), +107° (*c* 1.2, MeOH); IR ν_{max} (CH_2Cl_2) cm^{-1} 3425, 2970, 2940, 2880, 1698, 1460, 1380, 1105, 970; UV λ_{max}^{MeOH} nm end absorption; FD-MS *m/z* 737 ($M-SC_2H_5$), 443 (ABC, $C_{23}H_{39}O_8$), 271 (EF, $C_{15}H_{27}O_2S$).

Anal Calcd for $C_{43}H_{74}O_{11}S$ (799.12): C 64.7, H 9.3, O 22.0, S 4.0.
Found: C 64.8, H 9.4, O 21.9, S 4.2.

Sodium Salt: MP 158~164°C (dec); $[\alpha]_D^{22}$ +116° (*c* 1.13, $CHCl_3$), +102° (*c* 0.9, MeOH); IR ν_{max} (CH_2Cl_2) cm^{-1} 2980, 2950, 2880, 1600, 1465, 1390, 1240, 1120, 1100, 1075, 1040, 1025, 970, 950; ^1H NMR δ 1.23 (3H, t), 2.42 (2H, m, for CH_3CH_2S), 3.33 (3H, s, OCH_3).

Methyl Ester: FD-MS *m/z* 813 (MH^+, $C_{44}H_{77}O_{11}S$), 752 ($MH^+-SC_2H_5$), 541 (ABCD, $C_{29}H_{49}O_9$), 457 (ABC, $C_{24}H_{41}O_8$), 271 (EF, $C_{15}H_{27}O_2S$), 187 (F, $C_{10}H_{19}OS$).

Preparation of Mutalomycin (**1**) and 28-Epimutalomycin (**3**) from **5**

A solution of **5** (3 g free acid, 3.75 mmol) in 100 ml acetone was treated with Raney-nickel (30 ml aqueous suspension in 100 ml acetone - dimethylformamide, 85 : 15) for 1 hour with vigorous stirring at 25°C. Filtration and evaporation of the solvents under reduced pressure gave a yellow foam, which was converted into the sodium salt (2.77 g) by treatment with 1 N NaOH. TLC showed mainly two products. Chromatographic separation on Silica gel G (Merck, 190 g) with toluene - acetone - triethylamine (80 : 20 : 1) afforded a less polar product (1.18 g, 41%). It was crystallized twice from acetone yielding white crystals: MP 153~160°C (dec); $[\alpha]_D^{22}$ +95° (*c* 1.05, $CHCl_3$); identical with mutalomycin (**1**).

Methyl Ester: FD-MS *m/z* 791 ($M+23^+$), 703 ($M+23-C_4H_8O_2$), 672, 575, 393, 312 (DEF, $C_{18}H_{32}O_4$), 227 (EF, $C_{13}H_{23}O_3$), 144 (F, $C_8H_{16}O_2$).

The second product (0.775 g, 27%) crystallized from acetone to give white crystals: MP 180~188°C (dec); $[\alpha]_D^{22}$ +100° (*c* 1.08, $CHCl_3$); identical in all respects with 28-epimutalomycin (**3**) isolated from the fermentation broth.

Methyl Ester: FD-MS *m/z* 791 ($M+23^+$), 750 ($M-18$), 703, 671, 576, 449, 311, 227, 144.

Preparation of 29-Deoxymutalomycin (**6**)

Mutalomycin (10 g, 12.9 mmol) in 1 liter ethyl acetate and 20 ml acetic acid was hydrogenated in the presence of 10 g 10% palladium carbon at 22°C for 46 hours. The reaction mixture was filtered and the solvents evaporated *in vacuo*. The residue was converted into the sodium salt with 1 N NaOH by the extraction procedure. Crystallization from acetone yielded 6.65 g (67%) colorless crystals: MP 187~195°C (dec); $[\alpha]_D^{22}$ +85° (*c* 1.1, $CHCl_3$), +73° (*c* 0.97, MeOH); IR ν_{max} (CH_2Cl_2) cm^{-1} 2980, 2940, 2890, 1595, 1460, 1385, 1110, 1080, 1055, 1044, 970, 940, 875; electron impact (EI)-MS *m/z* 760 (M, $C_{41}H_{69}O_{11}Na$); FD-MS *m/z* 761 $(MH)^+$.

Anal Calcd for $C_{41}H_{69}O_{11}Na$ (760.98): C 64.7, H 9.1, O 23.1, Na 3.0.
Found: C 64.5, H 9.0, O 23.1, Na 2.7.

Methyl Ester: FD-MS *m/z* 775 $(M+23)^+$, 655, 296 (DEF, $C_{18}H_{32}O_3$), 211 (EF, $C_{13}H_{23}O_2$), 127 (F, $C_8H_{15}O$).

Preparation of 28-Epimutalomycin (**3**) and 2-Epimutalomycin (**2**) from *Streptomyces mutabilis*

During the isolation of the metabolites from a culture broth of *S. mutabilis* NRRL 8088 (7,500 liters) we detected on TLC in addition to mutalomycin (**1**) (Rf 0.52) a polar minor metabolite (Rf 0.4) (**3**) and a more polar second minor metabolite (Rf 0.31) (**2**). Chromatographic separation of the sodium salts and crystallization from acetone gave 7.2 g of **3**: MP 187~191°C (dec); $[\alpha]_D^{22}$ +104° (*c* 1.0, $CHCl_3$), +86° (*c* 1.0, MeOH); IR ν_{max} (KBr) cm^{-1} 2960, 2940, 2870, 1590, 1450, 1400, 1385, 1235, 1120, 970, 940; EI-MS *m/z* 776 (M, $C_{41}H_{69}O_{12}Na$); FD-MS *m/z* 778 (MH_2^+, 100%), 733, 228 (EF,

$C_{13}H_{24}O_3$), 144 (F, $C_8H_{16}O_2$), 126 (F$-H_2O$, $C_8H_{14}O$).

Anal Calcd for $C_{41}H_{69}O_{12}Na$ (776.98): C 63.4, H 9.0, O 24.7, Na 2.9.
Found: C 63.5, H 8.6, O 24.8, Na 3.0.

Chromatographic purification and crystallization from acetone yielded 1.2 g sodium salt of **2**: MP 166~177°C (dec); $[\alpha]_D^{22}$ +106° (*c* 1.0, $CHCl_3$), +84° (*c* 1.0, MeOH); FD-MS *m/z* 778 (MH_2^+, $C_{41}H_{71}O_{12}Na$), 734, 311 (DEF, $C_{18}H_{31}O_4$), 227 (EF, $C_{13}H_{23}O_3$), 144 (F, $C_8H_{16}O_2$).

Anal Calcd for $C_{41}H_{69}O_{12}Na$ (776.98): C 63.4, H 9.0, O 24.7, Na 2.9.
Found: C 63.2, H 8.3, O 24.9, Na 2.9.

Crystal Data for 28-Epimutalomycin (3)

The compound crystallized from acetone as colorless, monoclinic crystals. Crystal data: $C_{41}H_{69}O_{12}K$, formula weight 793.03; space groups $P2_1$ a=7.627(7), b=19.520(1), c=14.179(6), α=90.0, β=100.24(53), γ=90.0, V=2077.31A^3, D_{calc}=1.268 gcm^{-3}, Z=2, μ=16.03 cm^{-1}.

Intensities were measured on an Enraf-Nonius CAD-4 diffractometer using monochromated CuK_α radiation (θ<60°). There was no measurable cyrstal decay and no absorption corrections where applied. Of the 3198 measured reflections 3010 had I>2.5σ(I) and were considered observed.

The structure was solved by direct method using SHELX-86(1). All hydrogen atoms attached to carbon atoms were included in calculated positions, the hydrogen atoms of the hydroxyl groups where located from a difference Fourier map and included in fixed positions. The final R factor was 0.0352.

Crystal Data for 2-Epimutalomycin (2)

The compound crystallized from hexane as colorless, orthorhombic crystals. Crystal data: $C_{41}H_{69}O_{12}K\cdot 2H_2O$, formula weight 829.05 space group $P2_12_12_1$ a=12.795(2), b=15.059(3), c=23.397(3), V=4508.1A^3, D_{calc}=1.221 gcm^{-3}, Z=4, μ=15.29 cm^{-1}.

Intensities were measured on an Enraf-Nonius CAD-4 diffractometer using monochromated CuK_α radiation to (θ<60°). There was no measurable crystal decay and no absorption corrections were applied. Of the 3781 measured reflections 3488 had I>2.5σ (I) and were considered observed.

The structure was solved by direct methods using SHELX-86(1). All hydrogen atoms attached to carbon atoms were included in calculated positions, the hydrogen atom at O-29 was located from a difference Fourier map and included in fixed positions. The final R factor was 0.0576.

Fractional atomic coordinates and anisotropic temperature factors of the non-hydrogen atoms have been deposited and are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England.

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