# Aurantimycins, New Depsipeptide Antibiotics from *Streptomyces aurantiacus* IMET 43917

## Production, Isolation, Structure Elucidation, and Biological Activity

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Aurantimycins A (**1**), B (**2**) and C (**3**) were isolated from the mycelium of *Streptomyces aurantiacus* JA 4570 as new representatives of the azinothricin group of hexadepsipeptide antibiotics. Their structures were settled by X-ray diffraction analysis of crystalline aurantimycin A (**1**), high field homo- and heteronuclear 2D NMR experiments, high-resolution mass spectrometry and amino acid analysis. Aurantimycins are characterized by a new side chain containing fourteen carbon atoms. They display strong activity against Gram-positive bacteria and cytotoxic effects against L-929 mouse fibroblast cells.

In the course of a screening for new ionophoric compounds[1], *Streptomyces aurantiacus* IMET 43917 was found as the producer of antibacterial antibiotics displaying high activity against *Bacillus subtilis* ATCC 6633. Their effect on this test organism could be reduced by high potassium concentrations in the medium. Moreover, the antibacterial activity could be ascribed to a series of peptide structures, aurantimycins A (**1**), B (**2**) and C (**3**), which are formed by this strain in addition to pamamycin-621[2,3].

The new aurantimycins A (**1**), B (**2**) and C (**3**) (Figs. 1 and 2) are related to known hexadepsipeptides such as azinothricin[4], variapeptin[5], citropeptin[5], A 83586C[6], L-156,602[7], PD 124,895[8], PD 124,966[8] and IC101[9]. For these lipophilic antibiotics antibacterial and cytotoxic, as well as inhibitory effects on cellular adhesion[9] and C5a anaphylatoxin receptor binding[7] have been described. Here we report the isolation, structure elucidation and biological properties of aurantimycins A, B, and C, as new homologous representatives of the azinothricin family.

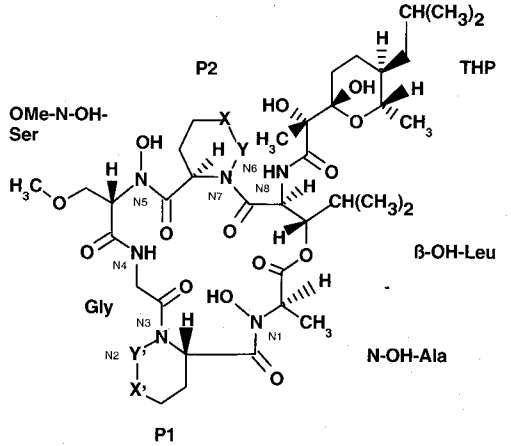
## Production and Isolation

The producing microorganism *Streptomyces aurantiacus* IMET 43917, isolated by Krassilnikov[10] in China, was obtained from the IMET strain collection (a subdivision of Deutsche Sammlung Mikroorganismen, Göttingen (DSM), Germany). It was classified as *Streptomyces aurantiacus* according to taxonomic studies[10] and deposited in the DSM culture collection, Göttingen, Germany, with the accession No. DSM 8818.

Stock cultures of IMET 43917 were suspended in glycerol (10%) - lactose (5%) solution and preserved by storage in liquid nitrogen. These suspensions served as inoculum for a medium containing (g/liter): glucose 15, soya bean flour 15, $CaCO_3$ 1, NaCl 5, $KH_2PO_4$ 1, pH 6.2 (prior to sterilization).

Inoculated 250 ml Erlenmeyer flasks containing 50 ml

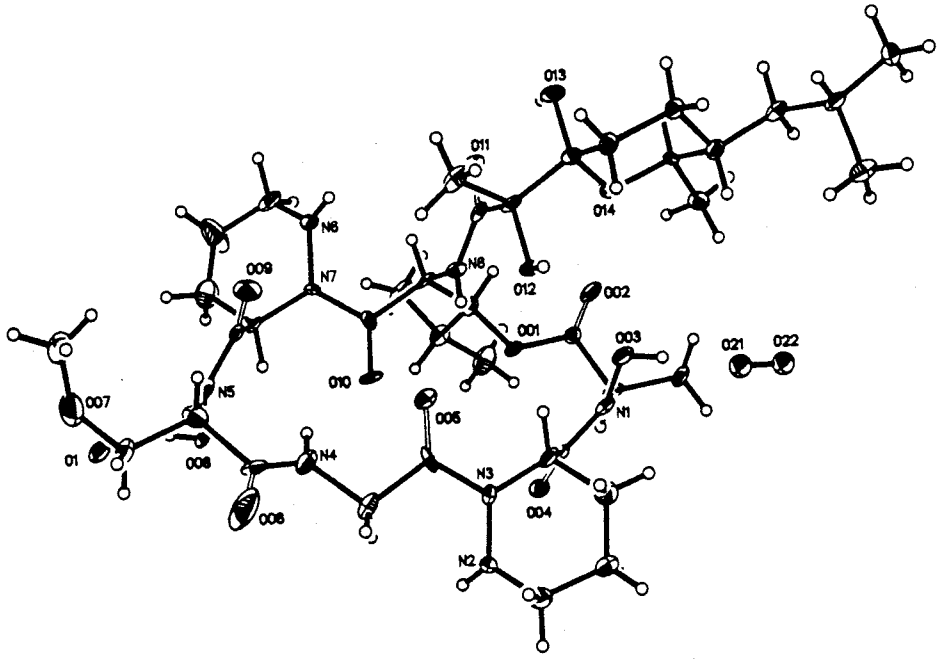
Fig. 1. Structures of aurantimycins A, B and C.




Aurantimycin A (**1**) X–Y=X′–Y′: $-CH_2-NH-$
B (**2**) X–Y: $-CH_2-NH-$; X′–Y′: $-CH=N-$
C (**3**) X–Y=X′–Y′: $-CH=N-$

THP: tetrahydropyranyl-tridecanoic acid; P1 and P2: piperazinic and dehydropiperazinic acid moieties, respectively (see Table 2).

Fig. 2. Molecular structure and thermal ellipsoids (20% probability) of aurantimycin A (**1**) as got by an X-ray single crystal structure analysis.




medium were incubated for 48 hours at 28 °C on a rotary shaker orbiting in a 10 cm circle at 150 rpm. The resulting culture was transferred into 400 ml volumes of the same medium in 2-liter shake flasks. After a further incubation period of 48 hours, the mycelium culture was used as 3% inoculum for a 150 liters fermentor. The fermentor medium contained (g/liter): glucose 20, soya bean 20, NaCl 5, $CaCO_3$ 3, pH 7.2 (prior to sterilization).

The well aerated culture was harvested after 96 hours of cultivation at 28°C, the mycelium cake extracted twice by five volumes of MeOH per volume of wet mycelium and the methanol evaporated *in vacuo*. The residue was extracted twice by 5 liter $CH_2Cl_2$, the organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. Crude material (40 g) was subjected to column chromatography on silica gel 60 using stepwise ethylacetate, acetone, and methanol as eluents. The acetone eluate containing the main activity was evaporated to a small volume and the aurantimycins precipitated by the addition of 300 ml *n*-hexane (yield 260 mg). Final purification was achieved by preparative isocratic HPLC using acetonitrile - water (83 : 17) and a 2 × 25 cm column (Nucleosil C7 RP18, 7 $\mu$m, 5 ml/minute, 210 nm, Macherey and Nagel). Yield: aurantimycin A (**1**, retention time 12.7 minutes) 66 mg; aurantimycin B (**2**, retention time 11.4 minutes) 85 mg; aurantimycin C (**3**, retention time 9.3 minutes) 3 mg. The latter component (**3**) was formed occasionally as a minor component of some few fermentations.

A concentrated solution of aurantimycin A in $CHCl_3$ - *n*-hexane (1 : 1) crystallized in the refrigerator to yield white needles suitable to X-ray diffraction studies.

## Structure Elucidation

Summarized in Table 1 are the physico-chemical properties of the aurantimycins A (**1**), B (**2**) and C (**3**) (Fig. 1). Positive ion FAB and EI mass spectra were recorded on an AMD 402 double focusing instrument of BE geometry (AMD Intectra, Harpstedt, Germany). Ions were produced by fast ion bombardment with a 12 KeV $Cs^+$ ion beam generated in a $Cs^+$ gun (liquid SIMS system, AMD Intectra). Peptide solutions were mixed with m-nitrobenzyl alcohol as matrix on the FAB probe tip. Exact mass measurements were performed using the peak matching technique (PEG as standard). Electrospray mass (ESMS) spectra (Fig. 3) were recorded on a quadrupole mass spectrometer Quattro (VG Biotech, Altrincham, U.K.). Molecular compositions of **1**, **2** and **3** as shown in Table 1 were inferred from accurate HRFAB-MS investigations. In comparison with aurantimycin A (**1**), the aurantimycins B (**2**) and C (**3**) exhibit two and four units, respectively, lower molecular weight. Additional support for the molecular formula of **1** was obtained by HREI-MS of the dimethyl derivative (see Experimental). Apparently, the aurantimycins A ~ C form a group of homologues differing only by the degree of saturation.

Chiral amino acid analysis revealed the presence of 2*S*,3*S*-hydroxyleucine and glycine in each compound. Moreover, formation of iron(III) complexes, as a general

Fig. 3. Negative ion mode ESMS spectra of aurantimycin A (**1** (a)) and aurantimycin B (**2** (b)).

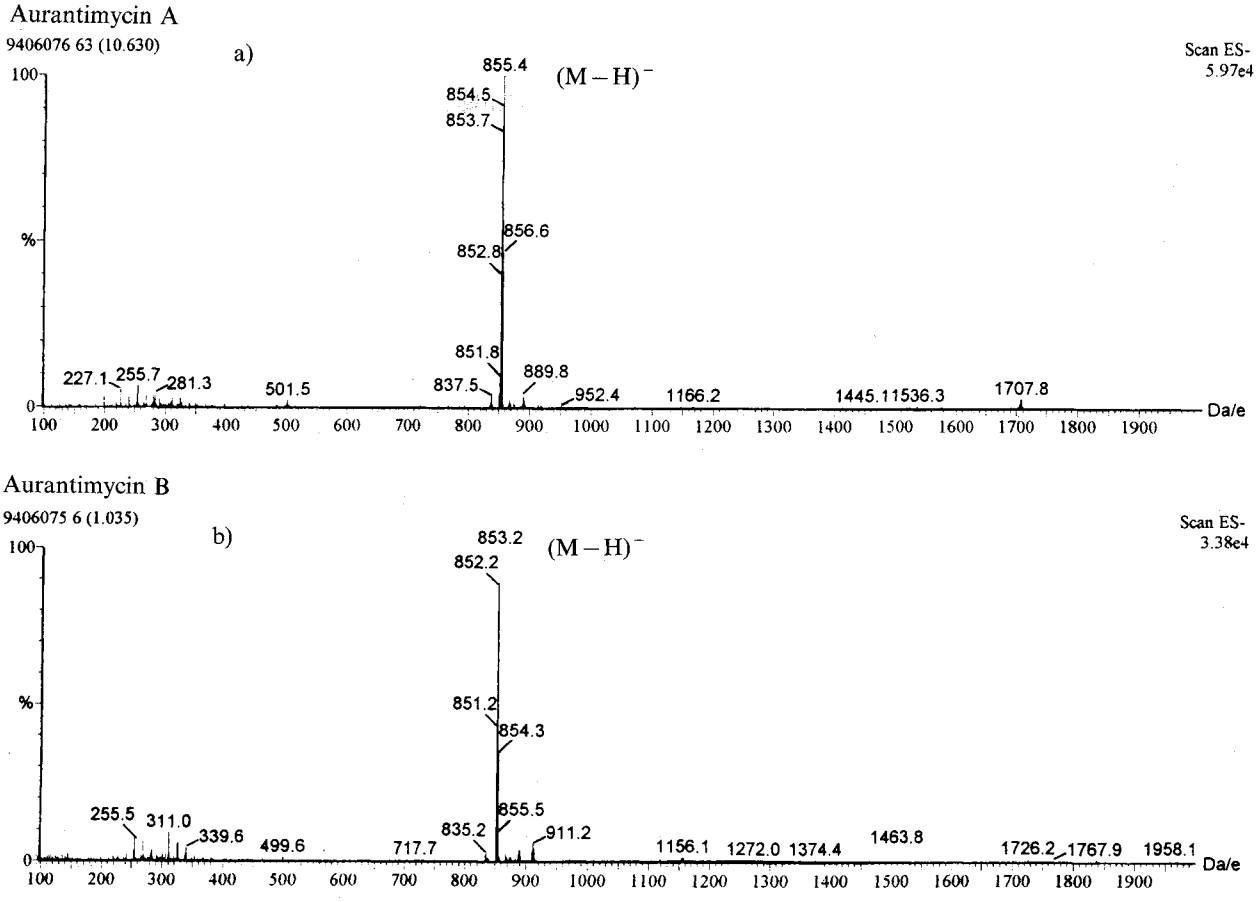



Table 1. Physicochemical properties of aurantimycins A (**1**), B (**2**), and C (**3**).

| Aurantimycin | A (**1**) | B (**2**) | C (**3**) |
|---|---|---|---|
| Appearance | White needles | White powder | White powder |
| Electrospray MS Neg. *m/z* | 855.1 $(M-H)^-$ | 853.1 $(M-H)^-$ | 851.2 $(M-H)^-$ |
| HRFAB-MS Neg. *m/z* | 855.4444 $(M-H)^-$ | 853.4350 $(M-H)^-$ | — |
| | 855.4464 (Calcd for $C_{38}H_{63}N_8O_{14}$) | 853.4308 (Calcd for $C_{38}H_{61}N_8O_{14}$) | — |
| Molecular weight | 856 | 854 | 852 |
| Molecular formula | $C_{38}H_{64}N_8O_{14}$ | $C_{38}H_{62}N_8O_{14}$ | $C_{38}H_{60}N_8O_{14}$ |
| MP | 197～198°C | 212～215°C | 205～208°C |
| $[\alpha]_D^{20}$ ($CHCl_3$/MeOH, 1:1) | +7.8° | +33.1° | — |
| Solubility Soluble: | MeOH; EtOH; $CHCl_3$, ethylacetate | MeOH; EtOH; $CHCl_3$, ethylacetate | MeOH; EtOH; $CHCl_3$, ethylacetate, |
| Insoluble: | *n*-Hexane, $H_2O$ | *n*-Hexane, $H_2O$ | *n*-Hexane, $H_2O$ |
| Color reaction: on TLC | Green staining by 2% vanillin in conc. $H_2SO_4$ | Green staining by 2% vanillin in conc. $H_2SO_4$ | Green staining by 2 %vanillin in conc. $H_2SO_4$ |
| Rf values on TLC (Silica gel Merck, $F_{254}$; $CHCl_3$/MeOH 97:3) | 0.85 | 0.78 | 0.62 |
| Retention time on HPLC (Encapharm C18 5 $\mu$, 4.6 × 250 mm acetonitril/$H_2O$; 83:17; 1 ml/minute, 210 nm, 23°C) | 5.7 | 4.9 | 4.2 |
| IR $\lambda_{max}$ ($cm^{-1}$) | 911, 1042, 1087, 1124, 1125, 1197, 1230, 1260, 1316, 1405, 1453, 1511, 1631, 1680, 1739, 2945, 3770, 3385 | 1007, 1043, 1100, 1122, 1153, 1198, 1231, 1254, 1311, 1430, 1436, 1508, 1648, 1652, 1739, 2955, 3270, 3385 | Not determined |

characteristic of *N*-hydroxamate structures, was proved by the increase of optical density at $\lambda_{max}$ 420 nm upon the addition of $FeCl_3$ to a methanolic solution of aurantimycin A, B, and C.

For X-ray diffraction analysis a crystal of dimensions $0.5 \times 0.12 \times 0.1\,mm^3$ was sealed in a Lindemann-glass capillary. 25 reflections with $2\theta > 6°$ were used to determine the cell parameters on a four circle computer controlled diffractometer (R 3 m/V, Siemens): $C_{38}H_{64}N_8O_{14} \cdot 2H_2O$, $M_r = 893.00$, monoclinic, $P2_1$, a = 15.06 (1), b = 10.009 (6), c = 16.245 (6) A, $\beta = 106.10$ (2)°, V = 2352 (4) $A^3$, Z = 2, $D_x = 1.26\,Mg\,m^{-3}$, $\lambda$(Mo K$\alpha$) = 0.71073 A°, $\mu = 0.1\,mm^{-1}$, F(OOO) = 968.0, T = 193 K.

12679 reflections ($-16 < h < 16$, $-11 < k < 11$, $-18 < l < 17$), 7004 unique and 4836 possessing structure factors $|F| > 1\sigma$ (F), were used for the structure analysis until the last refinement cycles. Direct methods were used to solve the phase problem[11] followed by refinement of the structure parameters by least-square methods (minimization of $(F_o^2 - F_c^2)^2$, weighting scheme: $w = 1/\sigma^2$ ($F^2$) according to the counting statistics). 581 parameters, coordinates of the H atoms were calculated according to the usual geometry (S = 0.83, R = 0.077 for 2399 unique diffractometer data ($>4\sigma$), wR = 0.204 for all unique data, 10 largest peaks in the difference map: 0.28...0.41 electrons/$A^3$). All calculations were done by a VAX station 3100 with the SHELXTL-PLUS and SHELXL[12] programs. Tables of further results are available as Supplementary Material†.

In the crystal (Fig. 2) there are quite strong hydrogen bonds between water molecules and the depsipeptide: 01 forms hydrogen bonds with O04 (2.90 Å) and 010 (2.66 Å) of the same and with O08 of another molecule (2.67 Å). The disordered water molecule 021/022 is joining O03 (2.60 E) and O06 (2.78 Å) of two different depsipeptide molecules. There is also a weak intermolecular hydrogen bond between O03 and O13 (3.07 Å). All the other nitrogen and oxygen atoms form intramolecular hydrogen bonds: O03···HO12 (2.97 Å), O4···HN2 (2.96 Å), O05···HN4 (2.70 Å), O05···HN8 (3.29 Å), O07···HO8 (3.43 Å), O09···HN6 (3.04 Å), O10···HN8 (3.01 Å), O11···HO13 (2.70 Å), O12···HN8 (2.65 Å), N4H···N5 (2.74 Å). A few of these are bifurcated. This system of intramolecular hydrogen bonds reveals that the ring system is rather rigid.

The 19-membered hexadepsipeptide ring of aurantimycin A (**1**) consists of the four amino acids alanine, glycine, serine and $\beta$-hydroxy-leucine (Fig. 2). The other two amino acids are piperidazine-6-carbonic acids (piperazinic acids), where the $\alpha$-carbon atoms have opposite configurations. This is also the case in other hexadepsipeptides[4~9]. Except for glycine, all of the nitrogen atoms of the amino acids are substituted: L-alanine and *O*-methyl-L-serine by a hydroxy group, and 2*S*,3*S*-hydroxyleucine by the tetrahydropyranyl derivative 2-hydroxy-2-(2-hydroxy-5-isobutyryl-6-methyl-tetra-hydropyran-2-yl)-propionic acid.

The absolute stereochemistry of aurantimycin A (**1**) as shown in Figs. 1 and 2, has been settled due to the presence of 2*S*-amino-3*S*-hydroxy-4-methylpentanoic acid (2*S*,3*S*-hydroxyleucine) in the acidic hydrolysate of aurantimycins A~C (**1~3**).

Further conclusive structural evidence has been provided by the NMR spectroscopic data (Table 2) on **1**, **2**, and **3**. All NMR spectra were recorded at 300 K on a Bruker AMX 600 spectrometer with sample concentrations of 70, 57 and 38 mmol for **1~3**, respectively. The complete $^1H$ and $^{13}C$ NMR assignments of **1~3** as well as the structure elucidation of **2** and **3** were performed by homo- and heteronuclear 2D NMR experiments. First the $^1H$ NMR assignments of different spin systems were carried out by COSY and phase sensitive TOCSY experiments. The second step was the assignment of the protonated carbons using the HMQC technique as well as the interconnection of the fragments by use of phase sensitive NOESY and HMBC spectra.

In the $^{13}C$ spectra of aurantimycin B (**2**) and aurantimycin C (**3**), one and two $CH_2$ signals, respectively, were replaced by $sp^2$-hybridized carbons ($\delta_c$ 144.6 ppm and 144.1/144.6 ppm, respectively) indicating that the difference from aurantimycin A could be ascribed to the presence of additional double bonds within these homologue structures. Location of these heterologous double bonds between C-5 and the neighbouring nitrogen of the piperazinic acid (Pip) moieties could readily be assigned due to the changed spin system in the COSY and TOCSY spectra. Thus **2** and **3** contain one and two dehydropiperazinic acid (dehydro Pip) moieties respectively. The position of the dehydropiperazinic acid of **2** within the molecular frame work was determined by consideration of NOE effects between the 2-H of Pip

† Lists of the anisotropic atomic displacement parameters, of bond lenghts and bond angles, of torsion angles, of H atom coordinates and of the structure factors have been deposited with the British Library Document Supply Centre als Supplementary Publication. Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, hester CH1 2HU, England.

Table 2. $^1H$ and $^{13}C$ NMR chemical shifts of the aurantimycins A (**1**), B (**2**) and C (**3**). ($CDCl_3$, $\delta$ in ppm relative to internal TMS, $J_{HH}$ in Hz). Abbreviations. P2 and P1: piperazinic acid or 2,3-dehydropiperazinic acid. (Pip, dehydro-pip), THP: substituted tetrahydropyranyl side chain.

| | | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|---|
| | | $\delta_C$ | $\delta_H$ ($J_{HH}$) | $\delta_C$ | $\delta_H$ ($J_{HH}$) | $\delta_C$ | $\delta_H$ ($J_{HH}$) |
| N-OH-Ala: | C-1 | 169.1 s | | 168.9 s | | 169.1 s | |
| | C-2 | 52.4 d | 5.37 q, 6.8 | 53.5 d | 5.37 q, 6.9 | 53.4 d | 5.34 q, 6.9 |
| | C-3 | 12.6 q | 1.37 d, 6.8 | 12.9 q | 1.37 d, 6.9 | 13.0 q | 1.35 d, 6.9 |
| P2: | NH | | 4.95 d, 11.3 | | | | |
| | C-1 | 170.3 s | | 170.7 s | | 170.6 s | |
| | C-2 | 48.5 d | 5.46 d, 4.2 | 49.0 d | 5.45 br | 50.7 d | 5.52 d, 5.1 |
| | C-3 | 23.0 t | 1.89 m, 2.25 m | 17.1 t | 2.01 m, 2.22 m | 17.3 t | 1.94 m, ~2.25 m |
| | C-4 | 20.1 t | 1.56 m, 1.63 m | 19.3 t | 2.14 m, 2.26 m | 19.5 t | ~2.16 m, ~2.27 m |
| | C-5 | 46.6 t | 2.83 m, 3.15 m | 144.6 d | 7.03 obsc | 144.6 d[a] | 7.03 obsc |
| Gly: | NH | | 6.98 br t, ~3 | | 7.03 br t, ~3 | | 6.92 br t, ~3 |
| | C-1 | 171.9 s | | 171.5 s | | 171.7 s | |
| | C-2 | 41.3 t | 4.03 dd, 18.7, 4.4 | 42.2 t | 4.26 dd, 18.5, 4.3 | 42.2 t | 4.10 dd, 18.9, 4.0 |
| | | | 4.61 dd, 18.7, 4.0 | | 4.64 dd, 18.5, 4.0 | | 4.85 dd, 18.9, 4.4 |
| OMe-N-OH-Ser: | C-1 | 167.8 s | | 168.3 s | | 168.1 s | |
| | C-2 | 57.2 d | 5.20 dd, 8.0, 5.9 | 57.5 d | 5.24 dd, 8.6, 5.3 | 57.0 d | 5.27 dd, 8.9, 5.1 |
| | C-3 | 67.3 t | 3.88 m, 3.96 m | 67.4 t | 3.87 m 11.0, 5.3 | 67.7 t | 3.84 dd, 10.6, 5.2 |
| | | | | | 4.00 dd, 11.0, 8.6 | | 3.99 dd, 10.6, 8.9 |
| | O-$CH_3$ | 58.5 q | 3.36 s | 58.8 q | 3.37 s | 59.0 q | 3.35 s |
| P1: | NH | | 4.74 br d, 11.8 | | 4.82 br d, 13.2 | | |
| | C-1 | 173.5 s | | 172.9 s | | 170.3 s | |
| | C-2 | 49.1 d | 5.49 br d, 4.3 | 50.6 d | 5.47 br | 50.1 d | 5.54 d, 5.0 |
| | C-3 | 24.5 t | 1.97 m, 2.28 m | 24.6 t | 1.95 m, 2.25 m | 18.3 t | 2.01 m, ~2.25 m |
| | C-4 | 21.3 t | 1.58 m, 1.62 m | 21.3 t | 1.57 m, 1.62 m | 19.7 t | ~2.16 m, ~2.27 m |
| | C-5 | 46.9 t | 2.78 m, 3.17 m | 47.1 t | 2.74 m, 3.19 m | 144.1 d[a] | 7.03, obsc |
| $\beta$-OH-Leu: | NH | | 7.19 br d | | 9.7 7.19 br d, 9.6 | | 7.47 br d, 9.3 |
| | C-1 | 171.8 s | | 170.9 s | | 170.0 s | |
| | C-2 | 45.9 d | 6.11 dd, 9.7, 4.3 | 46.4 d | 6.14 dd, 9.6, 4.3 | 47.4 d | 6.18 dd, 9.3, 4.1 |
| | C-3 | 78.7 d | 4.86 dd, 10.3, 4.3 | 78.9 d | 4.87 dd, 10.3, 4.3 | 79.9 d | 4.92 dd, 10.2, 4.1 |
| | C-4 | 29.6 d | 1.98 m | 29.9 d | 1.94 m | 30.3 d | 1.79 m |
| | C-5 | 17.9 q | 1.00 d, 6.8 | 18.1 q | 1.01 d, 6.8 | 18.6 q | 1.05 d, 6.8 |
| | C-6 | 19.4 q | 0.86 d, ~7 | 19.8 q | 0.87 d, ~7 | 19.3 q | 0.85 d, ~7 |
| THP | C-2 | 71.3 d | 3.71 m | 71.7 d | 3.74 m | 71.8 d | 3.74 m |
| | C-3 | 39.5 d | 1.02 m | 39.8 d | 1.05 m | 39.9 d | 1.04 m |
| | C-4 | 24.4 t | ~1.42 m, 1.66 m | 24.2 t | ~1.44 m, 1.67 m | 24.2 t | 1.45 m, 1.65 m |
| | C-5 | 27.1 t | 1.66 m, 1.74 m | 27.4 t | 1.65 m, 1.75 m | 27.5 t | 1.67 m, 1.78 m |
| | C-6 | 98.6 s | | 98.8 s | | 99.0 s | |
| | C-7 | 76.4 s | | 77.0 s | | 77.0 s | |
| | 7-$CH_3$ | 21.4 q | 1.55 s | 22.0 q | 1.54 s | 22.3 q | 1.48 s |
| | C-8 | 175.8 s | | 176.0 s | | 176.0 s | |
| | C-1′ | 40.8 t | ~0.95 m, 1.11 m | 41.0 t | ~0.95 m, 1.12 m | 41.1 t | ~0.94 m, 1.10 m |
| | C-2′ | 23.9 d | 1.67 m | 24.0 d | 1.62 m | 24.7 d | 1.63 m |
| | C-3′ | 21.3 q | 0.84 d, ~7 | 21.5 q | 0.83 d, 6.5 | 21.6 q | 0.88 d, 6.5 |
| | C-4′ | 24.0 q | 0.89 d, 6.6 | 24.2 q | 0.89 d, 6.8 | 24.3 q | 0.83 d, 6.6 |
| | C-1″ | 19.1 q | 1.13 d, 7.0 | 19.4 q | 1.15 d, 7.0 | 19.3 q | 1.05 d, 7.0 |

[a] Assignment exchangable

($\delta$ 5.41) and dehydro-Pip ($\delta$ 5.51) and the well resolved NOH-groups of N–OH–Ala ($\delta$ 9.31) and OMe–N–OH–Ser ($\delta$ 10.36) in the NOESY spectrum (recorded in DMSO-$d_6$).

The structures of aurantimycins A, B and C, as depicted in Fig. 1, suggest them to be new representatives of the azinothricin family of antibiotics featuring structural elements of both a depsipeptide and a polyether antibiotic[1]. They are distinguishable from the known piperazinic acid derivatives by the nature of the side chain and the constituent amino acids. Moreover, iron(III) complex formation in organic solvents, such as *e.g.* methanol ($\lambda_{max}$ 470 nm, reveals that aurantimycins and similar antibiotics could serve as a lipophilic carrier of trivalent iron within biological membranes. Aurantimycins share this property with the the recently published

ferrocins[13], but the latter contain catechol structures as iron-complexing subunits.

### Biological Activities

The cytotoxicity of aurantimycins A and B was examined against L-929 cells cultivated in Eagle's essential minimum medium. Both homologues caused a sudden change from nontoxic to lethal concentrations in the range of 3 to 12 ng/ml. The half maximum inhibitory concentration ($IC_{50}$) after 48, and respectively, 72 hours incubation amounted to 7 ng/ml for aurantimycin A and 6 ng/ml for aurantimycin B.

The $IC_{50}$ was defined as the concentration measured by the peapendiculas at the intersection of the concentration-effectivity curve of the aurantimycin treated wells with the half maximum optical density of controls without aurantimycin.

It could be proposed that formation of iron complexes contributes to the high cytotoxicity of the aurantimycins. Moreover, the formation of membrane pores[14] appears as another property of I, II and III which may contribute to cell-damaging activity.

Aurantimycins display narrow spectrum activity against Gram-positive bacteria but Gram-negative bacteria (*Escherichia coli*, *Pseudomonas* spec.) and fungi (*Aspergillus* spec., *Candida* spec.) were insusceptible (MIC $\gg$ 100 $\mu$g/ml). A particularly strong antibacterial effect of **1** was found on *Bacillus subtilis* (ATCC 6633 (MIC 0.02 $\mu$g/ml, *Micrococcus flavus* ATCC 10240 (MIC 0.08 $\mu$g/ml), *Micrococcus luteus* SG 125 A (MIC 0.08 $\mu$g/ml), *Staphylococcus aureus* SG 511 (MIC 0.040 $\mu$g/ml), *Staphylococcus aureus* 285 (MIC 0.013 $\mu$g/ml), *Streptococcus pyogenes* 303 (MIC 0.007 $\mu$g/ml), and *Streptococcus faecium* (MIC 0.025 $\mu$g/ml). Aurantimycins B and C show few differences in antibacterial spectrum, but two to four times higher concentrations were needed to cause the same antibacterial effect.

Moreover, aurantimycins A and B cause haemolysis of erythrocytes at concentration > 1.4 $\mu$g/ml. If both antibiotics had been administered intravenously to male NMRI mice the maximum tolerated single dose was 1 mg/kg body weight, but when given orally approximately 250 mg/kg.

## Experimental

Dimethyl derivative of aurantimycin A: 42 mg aurantimycin A dissolved in 150 ml methanol were treated dropwise with an excess of diazomethane as a freshly prepared solution in diethyl ether. The solvent was evaporated *in vacuo* and the residue further purified by column chromatography on silica gel 60 (0.063 ~ 0.1 mm, $CH_2Cl_2$ - MeOH 95 : 5). Yield 30.8 mg (Rf 0.85 on TLC (Merck Silica gel sheets, $CHCl_3$ - MeOH 95 : 5); waxy mass (EIMS: *m/z* 866.4729 $(M-H_2O)^+$; calcd. 866.4749 for $C_{40}H_{66}N_8O_{13}$; *m/z* 848.4612 $(M-2H_2O)^+$; calcd. 848.4643 for $C_{40}H_{64}N_8O_{12}$).

Amino acid constituents of aurantimycins were determined by acidic hydrolysis (6 N HCl, 24 hours) followed by chiral HPLC determination of the individual amino acids after derivatization by MARFEY's reagent[15,16] (Silica gel $RP_{18}$ 5 $\mu$m, Macherey and Nagel, Düren).

Cytotoxicity was determined after seeding $10^4$ L-929 cells in Eagle minimum essential medium into 96-wells microplates (COSTAR). Serial dilutions in 0.1 ml quantities of aurantimycin in culture medium were prepared in the plates previously. Incubation time was 48 and 72 hours at a temperature of 37°C in a humidified atmosphere with 5% $CO_2$. At the end of the experiments cells were stained with methylene blue[17] after fixation by glutaraldehyde. Thereafter, methylene blue was dissolved by 0.33 N HCl in isopropanol (0.2 ml) followed by a gentle washing procedure. Optical density was measured with a microplate reader (Dynatech 7000) at 630 nm in single modus against one well with medium but without cells, stained by the same methods as the treated wells.

Antibacterial activity was examined by the serial agar dilution method using Mueller-Hinton agar (Difco) for antimicrobial antibacterial tests with incubation at 37 °C for 18 hours and a nutrient agar for antifungal assays with incubation at 27°C for 42 hours.

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