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Isolation and structure elucidation of vancoresmycin—a new antibiotic from *Amycolatopsis* sp. ST 101170

Cordula Hopmann,[a,*] Michael Kurz,[a] Mark Brönstrup,[a] Joachim Wink[a] and Dominique LeBeller[b]

[a]*Aventis Pharma Deutschland GmbH, LG Natural Products Research, H 780, 65926 Frankfurt a. M., Germany*
[b]*Aventis Pharma France, 111 Route de Noisy, 93235 Romainville cedex, France*

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Abstract—A new antibiotic, active against Gram-positive bacteria, has been isolated from the fermentation broth of the actinomycete *Amycolatopsis* sp. ST 101170. The structure of the compound named vancoresmycin, a tetramic acid derivative with a highly oxygenated long alkyl chain, was elucidated by extensive spectroscopic studies and derivatization.

Infections caused by emerging resistant strains against the antibiotics vancomycin and teicoplanin are becoming a serious problem. In an effort to identify new natural products with antimicrobial activity the actinomycete strain ST 101170 (DSM 12216), a microorganism of the genus *Amycolatopsis*, was found to produce an inhibitor named vancoresmycin (**1**) with potent activity against vancomycin resistant strains like *Enterococcus* spp.[1] In this paper, we report on the isolation, structure elucidation and biological activity of **1** (Fig. 1).

For the isolation of the bioactive principle the mycelium of the culture broth (200 L) was exhaustively extracted with methanol (30–40 L) and the extract was concentrated in vacuo (10:1) to obtain a colorless precipitation, which was filtered off to yield 30–50 g crude material (30% purity). This material was chromatographed on Fractogel® TSK HW-40 solid support in methanol (70% purity). Final purification on a silica gel column (Gradient: CH_2Cl_2:MeOH 9:1 to CH_2Cl_2:MeOH+1% NEt_3 3:1) led to a white powder of **1** (yield for the two separations: 50%).

Vancoresmycin (**1**) showed UV maxima in methanol at 234 nm (log ε 4.39) and 280 nm (log ε 4.29). The IR spectra indicated the presence of hydroxyl groups (3384 cm^{-1}) and carbonyl functionalities (1674.5 and 1615.7 cm^{-1}). A mass spectrometric characterization of **1** was performed using an FTICR instrument (Bruker APEX III, 7T) equipped with an external ESI-source. The accurate mass of $(\mathbf{1}+H)^+$ was determined to be $m/z=$ 1343.8934 amu, which is in agreement with the theoretical mass of 1343.8926 amu calculated for $C_{71}H_{127}O_{21}N_2$ (0.6 ppm deviation). Based on extensive NMR studies including DQF-COSY, TOCSY, ROESY, HMQC and HMBC experiments, recorded in MeOD-d_4, three large substructures could be assigned. The first fragment covers carbons C-8″ to C-14. The carbon chemical shifts of C-1 (195.36 ppm), C-2″ (174.52 ppm), C-3″ (100.31 ppm) and C-4″ (183.80 ppm) suggest the presence of a tetramic acid moiety.

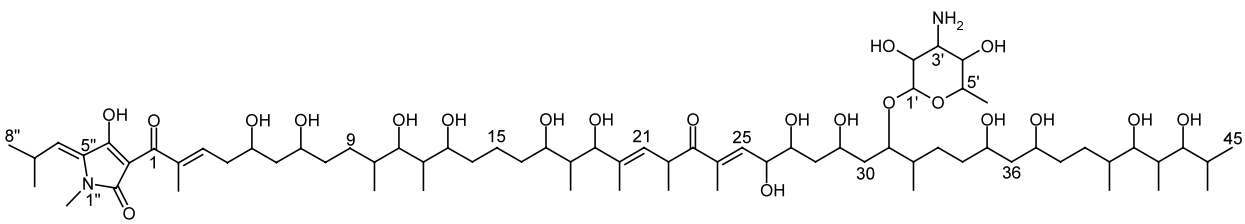

Figure 1. Structure of vancoresmycin (**1**).

* Corresponding author. Tel.: +49-69-30517914; fax: +49-69-305-20521; e-mail: cordula.hopmann@aventis.com

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The low field shift of C-4″ indicates an *exo* double bond between C-5″ and C-6″ as it is observed for the antibiotic magnesidin A.[2] The second fragment includes carbons C-16 to C-33. As shown by correlations in the HMBC spectrum (C-31/H-1′ and C-1′/H-31) a carbohydrate moiety is connected to C-31. The sugar carries an amino function in 3′-position and contains a methyl group in 6′-position. According to the homonuclear coupling constants it was identified as mycosamin.[3] The third substructure comprises carbons C-39–C-45 and represents a highly oxygenated alkyl chain with a unique substitution pattern. Due to spectral overlap it was not possible to connect the three substructures and add the remaining fragments of the molecule. To overcome this problem vancoresmycin was acetylated by treatment with acetanhydride in pyridine. The ratio of acetylation was monitored by LC–MS. After repetitive reaction a product was isolated incorporating 15 acetyl groups. The acetylation led to a much better dispersion of most proton and carbon signals including C-4–C-45 and allowed the assignment of the remaining fragment C-34 to C-38 and the CH_2-group in position 15. For the region of the tetramic acid moiety a significant line broadening was observed. The C–C double bonds between C-2–C-3, C-20–C-21 and C-24–C-25 have *trans* configuration as indicated by weak ROEs between the olefinic protons (H-3, H-21 and H-25) and the corresponding methyl groups (2-Me, 20-Me and 24-Me, respectively). A complete proton and carbon assignment in MeOD-d_4 is given in Table 1.

Table 1. ^1H and ^{13}C assignments of vancoresmycin (**1**) in MeOH-d_4 on a Bruker DRX-600 at 300 K

C-No.	δ_H	δ_C	C-No.	δ_H	δ_C	C-No.	δ_H	δ_C
1		195.36	20-Me	1.67	12.75	40-Me	0.86	12.75
2		141.66	21	5.36	128.35	41	3.53	80.19
2-Me	1.82	13.98	22	4.20	41.01	42	1.69	39.54
3	5.75	129.78	22-Me	1.14	17.77	42-Me	0.77	13.64
4	2.29	37.53	23	–	206.04	43	3.40	82.19
5	3.90	71.28	24	–	138.52	44	1.86	31.20
6	1.67/1.62	44.53	24-Me	1.82	12.75	44-Me	0.85	14.76
7	3.77	71.83	25	6.61	142.09	45	0.97	20.83
8	1.61/1.44	36.28	26	4.31	73.26	1′	4.59	103.48
9	1.54/1.33	31.34	27	3.79	72.47	2′	3.83	72.03
10	1.61	36.28	28	1.49	42.39	3′	2.48	57.61
10-Me	0.85	12.70	29	3.96	65.82	4′	3.08	75.08
11	3.40	77.82	30	1.52/1.42	39.35	5′	3.21	74.66
12	1.73	42.71	31	3.86	81.67	6′	1.27	18.29
12-Me	0.77	11.91	32	1.94	38.60	1″-Me	3.22	27.78
13	3.79	75.08	32-Me	0.92	15.04	2″	–	174.52
14	1.55/1.32	33.36	33	1.49/1.16	30.18	3″	–	100.31
15	1.62/1.32	23.51	34	1.57/1.38	36.61	4″	–	183.80
16	1.48	36.52	35	3.75	72.03	5″	–	136.51
17	3.58	73.85	36	1.60/1.54	44.90	6″	5.35	116.05
18	1.63	41.30	37	3.74	72.03	7″	3.07	26.50
18-Me	0.88	8.10	38	1.43	36.52	7″-Me	1.09	24.46
19	3.98	80.53	39	1.43	31.20	8″	1.09	24.46
20	–	138.72	40	1.65	36.40			

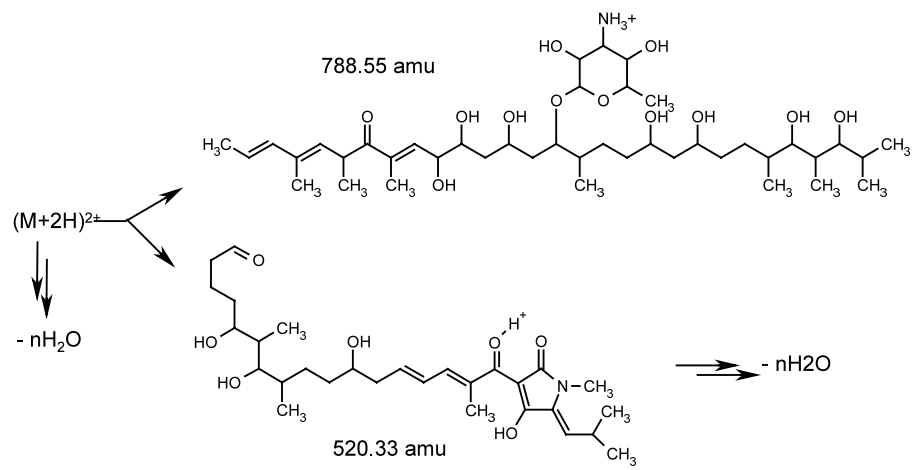

Figure 2. MS/MS-fragmentation pathways of vancoresmycin (**1**) in positive mode (ESI).

The MS/MS-fragmentation pathways of $(\mathbf{1}+2H)^{2+}$ and of $(\mathbf{1}\text{-}H)^{-}$ were studied by SORI-CID.[4] In addition to multiple loss of water a distinct preference for bond cleavage between C-17 and C-18 yielding fragments at 520.33 and 788.55 amu via a retro-aldol type reaction has been observed in the positive mode (Fig. 2). In the negative mode, the proposed ion structures presented in Fig. 3 are presumably formed via intramolecular esterification, elimination and retro-aldol reactions. All MS data are fully consistent with the NMR-derived structure.

Vancoresmycin (**1**) exhibited potent antibacterial activity against Gram-positive bacteria, including *Staphylococcus aureus* and *Enterococcus faecalis* as shown in comparison to vancomycin in Table 2. No inhibition effect against Gram-negative bacteria and no antifungal activity has been observed.

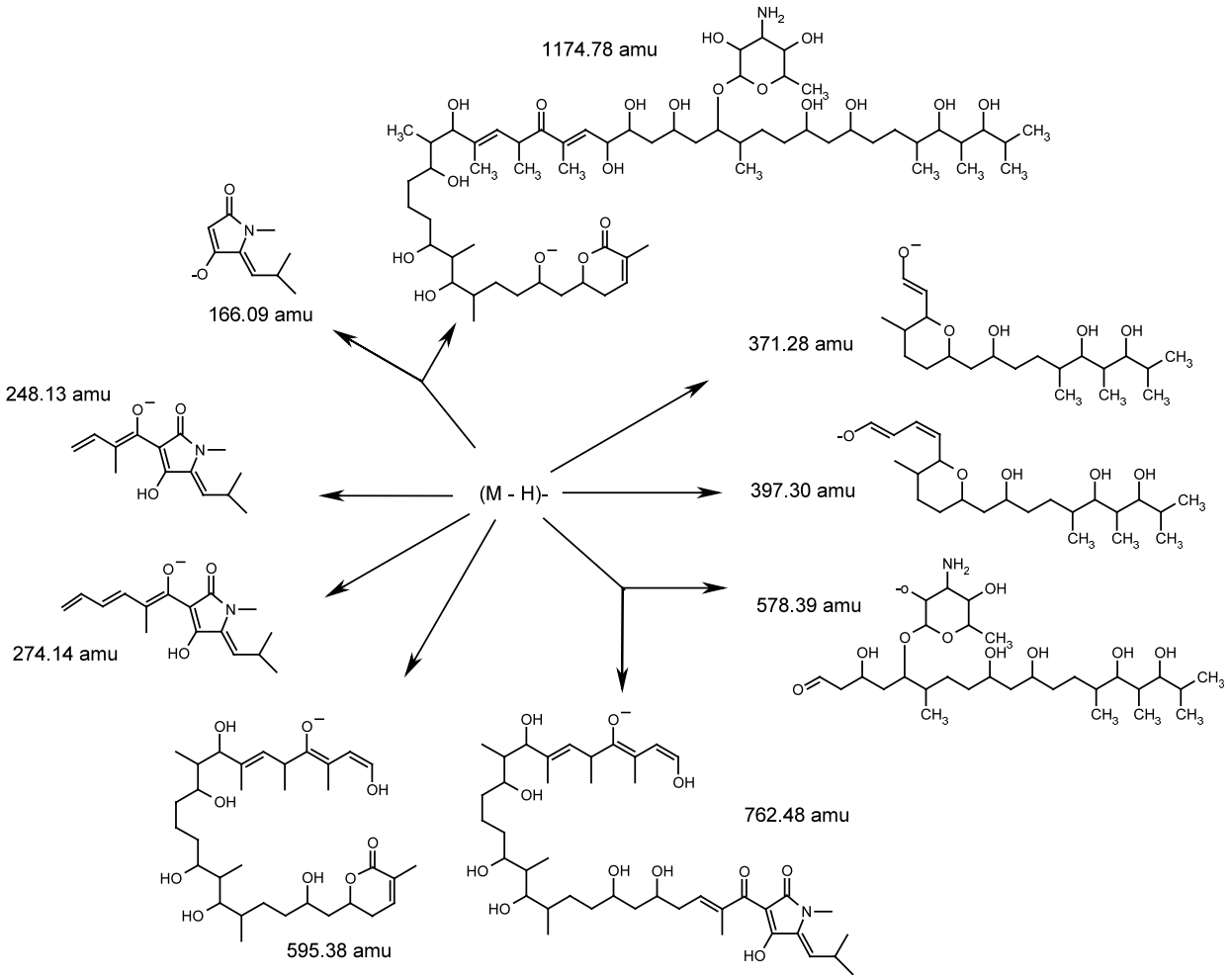

Figure 3. MS/MS-fragmentation pathways of vancoresmycin (**1**) in negative mode (ESI).

Table 2. MIC (mg/l) of vancoresmycin (**1**) (micromethod in broth)

Gram+strains	CODE		Vancoresmycin	Vancomycin
S. aureus	011HT3	oxa S ery S	≤0.04	0.3
S. aureus	011HT18	ATCC 13709 Smith	≤0.04	0.3
S. epidermidis	012GO42	oxa R	≤0.04	1.2
S. pyogenes	O2A1SJ1	van S ery Rc	≤0.04	0.15
S. pneumoniae	030BI2	ery R	≤0.04	0.15
E. faecium	O2D3HT12	tei R van R ery R tet R	0.08	>40
E. faecium	O2D3IP2	tei R van R ery R tet R	0.08	>40
E. faecium	O2D3HM3	nov S van A ery R tei R	≤0.04	>40
E. faecalis	O2D2HM9	nov R van B ery R tei S	0.6	>40
E. faecalis	O2D2UC5	ATCC 29212 nov R	0.3	2.5
E. faecalis	O2D2HT10	nov R van S tet R	0.3	0.6

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4. Although the mass accuracy of the FTICR was found to be lower (~10 ppm) in our MS/MS experiments than in a simple MS scan, a safe identification of important fragment ion compositions was possible in all cases. Low-mass and/or low-abundance fragments are not discussed here.