New 4-Oxovancosamine-containing Glycopeptide Antibiotics from *Amycolatopsis* sp. Y-86, 21022

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Balhimycin is a recently introduced glycopeptide antibiotic which was isolated from *Amycolatopsis* sp. Y-86, 21022, DSM 5908[1]. The characteristic structural feature of this new antibiotic is a 4-dehydrovancosamine sugar unit[2], which is mainly present as ketone hydrate. Balhimycin (**5**) exhibits antibacterial activity *in vitro* and *in vivo* against methicillin-resistant *Staphylococcus aureus* (MRSA) strains and, in addition, is potent against many anaerobic organisms. In order to become acquainted with the biochemical synthesis performance of *Amycolatopsis* sp. Y-86, 21022 and to discover new glycopeptide components, we examined nutrient broths of balhimycin producers.

Cultures of *Amycolatopsis* sp. Y-86, 21022 as described by S. R. NADKARNI *et al.*[1] served as starting material. By the adsorption resin technique on Diaion HP-20, the antibacterial compounds were desalted and concentrated. Their separation was carried out on an SP Spherodex M cation exchanger (Sepracor/IBF, France), while the final purification of the components was accomplished by means of preparative HPLC in a 0.05% trifluoroacetic acid/acetonitrile system and crystallization from water/ethanol (Scheme 1).

The most important data on the compounds isolated are summarized in Table 1. The glycopeptide **1** was identified as desvancosamine vancomycin[3] (**1**) with the aid of hydrolysis products and by degradation of commercial vancomycin. Various culture filtrates contain from 1 to over 5% ureidobalhimycin (**2**) related to the main product balhimycin. It has been found that this product can also be produced semisynthetically from balhimycin and KNCO; the crystals obtained by this means permitted the elucidation of the X-ray structure[4]. The reaction of 4-oxovancosamine with KNCO, which takes place easily, is a characteristic feature of balhimycin and related compounds. In the presence of this sugar unit, a reaction with the reagent always occurs, and this reaction can be used analytically. Compound (**3**) does not undergo a transformation with KNCO and, in fact, glucose was detected as the only sugar component. In addition to the hydrolysis products aspartic acid and N-dimethylleucine, which were identified by GC-MS, the MS, HR-MS and ^{1}H NMR spectra showed the identity to the well-known glycopeptide antibiotic M43C[5]. The glycopeptide (**4**), which occurred only in traces, is identical to the deglucobalhimycin described by S. CHATTERJEE *et al.*[2]. The hydrolysis products of **6** were glucose, rhamnose, and **4** and **5**. This proves that **6** is rhamnosyl balhimycin. From the fragmentation in the FAB-MS: 1594 $(M+H)^+$ (100%), 1448 $(M+H-\text{rhamnose})^+$ (20%), 1286 $(M+H-(\text{rhamnose}+\text{glucose}))^+$ (48%), the linking of the desoxy-sugar with glucose results, whereby the binding site on the glucose remains

Scheme 1. Isolation and purification of glycopeptides from *Amycolatopsis* sp. Y-86, 21022.

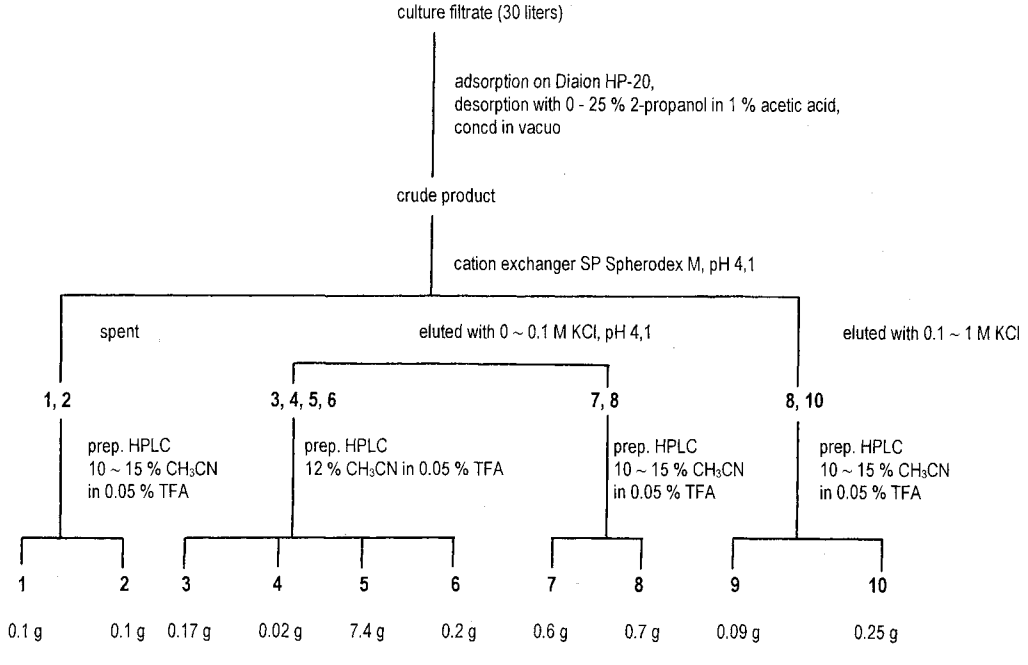


Table 1. Physico-chemical properties of glycopeptides from *Amycolatopsis* sp. Y-86,21022.

Compound	Found $(M+H)^+$	Empirical formula	M.W. Chem.	Rf† minutes	Rf†† of ureid minutes	Hydralysis products
Desvancosamine-vancomycin[3] (**1**)	1305.3[m]	$C_{59}H_{62}Cl_2N_8O_{22}$	1305	9.1	—	asp, (D)-N-methyl-leu*
Ureido-balhimycin[4] (**2**)	1491.1[a]	$C_{67}H_{74}Cl_2N_{10}O_{25}$	1490.3	—	13.1	asp, (D)-N-methyl-leu*
M 43 C[5] (**3**)	1319.333[m]	$C_{60}H_{64}Cl_2N_8O_{22}$	1320.1	14.4	—	asp, N-dimethyl-leu*
Degluco-balhimycin (**4**)	1284.4[m]	$C_{60}H_{63}Cl_2N_9O_{19}$	1285.1	22.6††	18.0††	asp, (D)-N-methyl-leu*
Balhimycin (**5**)	1447.8[m]	$C_{66}H_{73}Cl_2N_9O_{24}$	1447.3	9.8	13.1	asp, (D)-N-methyl-leu*
Rhamnosyl-balhimycin (**6**)	1594[a]	$C_{72}H_{83}Cl_2N_9O_{28}$	1593	8.3	9.5	glucose, rhamnose, **4**, **5****
Methyl-balhimycin (**7**)	1462.5[a]	$C_{67}H_{75}Cl_2N_9O_{24}$	1461.3	14.4	19.0	asp, N-dimethyl-leu*
Demethyl-balhimycin (**8**)	1432.5[m]	$C_{65}H_{71}Cl_2N_9O_{24}$	1433.3	8.6	10.4	asp, (D)-leu*
Dechloro-balhimycin V (**9**)	1520.3, 1538.1, 1556.7[a]	$C_{73}H_{86}N_{10}O_{26}$	1519.5	8.0	9.0	4-oxovancosamin, glucose, $C_{60}H_{67}N_9O_{19}$**
Balhimycin V (**10**)	1588, 1606, 1624[a]	$C_{73}H_{84}Cl_2N_{10}O_{26}$	1588.4	9.9	12.2	4-oxovancosamin, **5** glucose, **4****

[a] average $(M+H)^+$.
[m] monoisotopic $(M+H)^+$.
† HPLC-conditions: 14% acetonitrile in 0.1% TFA, nucleosil 100-5 C18 AB as stationary phase.
†† 19% acetonitrile in 0.1% TFA.
* 20% HCl, 105°C, 20 hours.
** 4n TFA, room temperature, 5~15 hours.

Table 2. ^{13}C and ^1H NMR data of 4-oxovancosamine in D_2O in comparison to that of 4-epi vancosamine.

^{13}C position	4-Oxovancosamine (ppm)		4-Epi vancosamine (ppm) in dihydrobalhimycin
	4	**5**	
1	94.2	92.6	93.3
2	42.8	40.2	39.1
3	61.7	61.0	57.5
4	95.0	95.3	75.6
5	73.5	68.4 (74.8)	67.0
6	14.5	14.7	17.9
3-CH_3	21.2	23.4	18.9
^1H position			
H-1	5.00	4.96	4.99
H-2	2.46	2.41	2.47
H-2′	2.33	2.33	2.23
H-4	—	—	3.43
H-5	3.97	3.97	3.78
Methyl-6	1.31	1.30	1.37
Methyl-3′	1.68	1.68	1.61

unclarified. The molecular formula $C_{67}H_{75}C_{12}N_9O_{25}$ of compound **7** corresponds to a higher homologue of balhimycin and, in fact, N,N-dimethylleucine was unequivocally identified by GC-MS as a constituent of the alkaline hydrolysate. Data from the ^1H NMR and ^1H-^{13}C wide-range correlations verify this result, as does the ^{13}C signal (43.9 ppm) of the N,N-dimethyl group. The remaining ^{13}C signal batch corresponds with that of balhimycin[2], consequently **7** must be the N,N-dimethylleucyl derivative. On the other hand, **8** is a norbalhimycin. The amino acid analysis yielded leucine in place of N-methylleucine, the physical and chemical properties of both antibiotics otherwise were comparable.

The FAB-MS of the more basic compound **10** showed, in addition to the molecular peak $M+H^+ = m/z$ 1588 (55%), two hydrate signals: 1606 (100%) and 1624 (35%) which indicate two 4-oxovancosamine units. For partial hydrolysis, **10** was left standing in 90% TFA at room temperature for 46 hours. The reaction mixture was subsequently concentrated in the vacuum and separated on a Fractogel EMD SO_3 cation exchanger (E. Merck) into the products glucose, 4-oxovancosamine, **5** and **4**. In order to clarify the linking of the amino sugar with balhimycin, **10** was reduced with $NaBH_4$. This reagent leads selectively to the conversion of the 4-oxo-amino sugar to 4 epivancosamines and, thereby, to A 82846 B which is known from the literature[6]. The NMR comparison with an authentic sample proved the identity of both glycopeptides. The structure of this glycopeptide is therefore deduced as **10**. Finally, product **9** which likewise contains two 4-oxovancosamine units: MS m/z 1520.3 $(M+H)^+$, 1538.1 $(M+H+H_2O)^+$, 1556.7 $(M+H+2H_2O)^+$, yielded on hydrolysis glucose, 4-oxovancosamine and a pseudoaglycon $C_{60}H_{67}N_7O_{19}$ (HR-MS: m/z 1218.467, calculated: 1218.463). The amino acid analysis showed the presence of aspartic acid and N-methylleucine. The palladium-catalyzed dehalogenation of **10** with triethylammonium formate in accordance with G. A. PETERSON *et al.*[7] resulted in **9**.

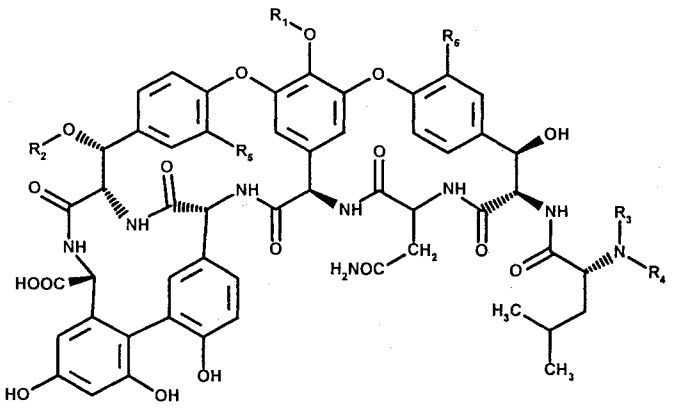
As a result of the low stability of 4-oxovancosamine, the isolation of the released **11** failed in the neutral media. The presence of the monoacetate could only be detected chromatographically and by means of ES+MS which was obtained in glacial acetic acid/TFA anhydride: m/z 220.1 $(M+H_2O+H)^+$ (38%), 202 $(M+H)^+$ (12%) corresponding to $C_9H_{15}NO_6$), 141.9 $(MH-2H_2O-CH_2CO)^+$ (100%). Under acidic conditions, however, on reversed phase with 0~10% acetonitrile in 0.1% TFA as eluent, sufficient material for the NMR investigations could be obtained from the hydrolysate of **10**, subsequent to ion-exchange chromatographic enrichment. The

Table 3. Comparative potency (MIC, μg/ml)[a] of glycopeptides isolated from cultures of *Amycolatopsis* sp. Y-86, 21022.

Organism	1	2	3	4	5	6	7	8	9	10
Staphylococcus aureus SG 511	1.5	0.8	3.1	0.2	0.2	0.2	0.4	0.1	0.8	0.4
Staphylococcus aureus 285	3.1	1.5	3.1	0.2	0.4	0.8	0.4	0.1	0.4	0.4
Staphylococcus aureus 503	1.5	1.5	3.1	0.1	0.2	0.2	0.4	0.05	0.2	0.1
Streptococcus pyogenes 308 A	1.5	0.8	3.1	0.1	0.1	0.2	0.4	0.05	0.4	0.05
Streptococcus pyogenes 77 A	1.5	0.8	3.1	0.1	0.1	0.2	0.4	0.05	0.4	0.05
Streptococcus faecium D	1.5	1.5	3.1	0.2	0.4	0.4	0.8	0.2	0.4	0.2
Escherichia coli TEM	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Peptostreptococcus anaerobius	n.t	6.2	50	0.4	12.5	n.t.	0.8	25	n.t.	0.2
Propioni acnes 6919	n.t.	0.8	6.2	0.2	0.4	n.t.	0.4	0.8	n.t.	0.2
Propioni acnes 6922	n.t.	1.5	6.2	0.2	0.4	n.t.	0.4	0.4	n.t.	0.1
Clostridium tetani ATCC 19406	n.t.	12.5	50	0.4	25	n.t.	3.1	50	n.t.	0.2
Clostridium perfringens 194	n.t.	0.4	6.2	0.1	0.2	n.t.	1.5	0.2	n.t.	0.1

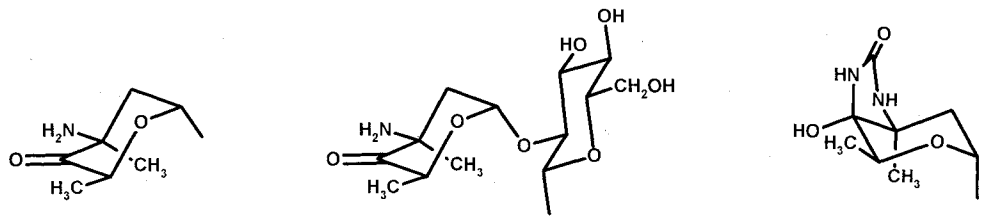
[a] Agar dilution method: Nutrient agar; 10^6 cfu/ml.
n.t.: Not tested.

Fig. 1. Structural assignments proposed for balhimycin-related compounds isolated from *Amycolatopsis* sp. Y-86, 21022.



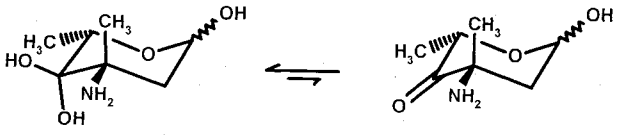
Compound	Compound No.	R_1	R_2	R_3	R_4	R_5	R_6
Devancosamine-vancomycin	1	Glc	H	H	CH_3	Cl	Cl
Ureido-balhimycin	2	Glc	Urvcn	H	CH_3	Cl	Cl
M 43 C	3	Glc	H	CH_3	CH_3	Cl	Cl
Degluco-balhimycin	4	H	Ovcn	H	CH_3	Cl	Cl
Balhimycin	5	Glc	Ovcn	H	CH_3	Cl	Cl
Rhamnosyl-balhimycin	6	Rha-glc	Ovcn	H	CH_3	Cl	Cl
Methyl-balhimycin	7	Glc	Ovcn	CH_3	CH_3	Cl	Cl
Demethyl-balhimycin	8	Glc	Ovcn	H	H	Cl	Cl
Dechloro-balhimycin V	9	Ovcn-glc	Ovcn	H	CH_3	H	H
Balhimycin-V	10	Ovcn-glc	Ovcn	H	CH_3	Cl	Cl

Glc: glucosyl-1, Ovcn: 4-oxovancosaminyl-1, Ovcn-Glc: 4-oxovancosaminyl (1 → 2)-glucosyl-1, Rha-glc: rhamnosyl (1 → 2)-glucosyl, Urvcn: ureido-vancosaminyl-1



Ovcn : 4-oxovancosaminyl -1 Ovcn-glc : 4-oxovancosaminyl(1-2)-glucosyl Urvcn : ureido-4-oxovancosaminyl -1

Fig. 2. Ketone/hydrate equilibrium of 4-oxovancosamine (**11**).



analysis of the ^1H NMR spectrum yielded 4 equilibrium products of the amino sugar in the D_2O solution. On the basis of the relative intensities which can be estimated at 1-H and assuming a largely intact chair formation, the following proportions can be determined:

26% α-anomeric ketone hydrate
63% β-anomeric ketone hydrate
8% β-anomeric ketone
3% α-anomeric ketone.

On account of the low proportions of the ketone, its signal could not be identified in the ^{13}C NMR spectrum. Detection of the ketone is therefore not established. However it can, unquestionably be deduced that the 4-oxovancosamine in solution is primarily (~89%) present as ketone hydrate.

Consequently, **11** is the only amino sugar which has been found in the balhimycin components. There was no indication of the existence of vancosamine[3], which frequently occurs in glycopeptides, or of 4-epivancosamine[7]. While the basic glycopeptide structure—the heptapeptide core—is common to all components, only the nature and extent of the glycosylation, the methylation of the leucine nitrogen, and the degree of the aromatic hydrocarbon halogenation vary.

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