

Resomycins A~C: New Anthracyclinone Antibiotics Formed by a Terrestrial *Streptomyces* sp.

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During the screening of Actinomycetes for novel bio-active components, we found that the terrestrial *Streptomyces* sp. isolate GW71/2497 produced large quantities of chartreusin^{1,2}, which precipitated during the concentration of the extract and was responsible for the high activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Streptomyces viridochromogenes* (Tü 57). On a soybean flour/mannitol medium instead of malt extract/yeast extract/glucose, the strain produced a totally different metabolic pattern: In addition to the metabolites detected previously, the TLC showed nonpolar yellow and orange zones giving a yellow or orange fluorescence under UV at 366 nm and colour reactions with sodium hydroxide characteristic for *peri*-hydroxyquinones. The work-up of the extract resulted in the isolation of several known compounds and of three new anthracyclinone antibiotics **2~4** which we named resomycin A~C. In this paper we report the taxonomy of the producing strain, the structure elucidation of **1a~4** and on the biological activity of these compounds.

Fermentation and Isolation

The strain was fermented in the usual manner in a 20 litre jar fermentor at 28°C for 72 hours. The total culture broth was exhaustively extracted with ethyl acetate, and the yellowish brown *semisolid* residue was pre-separated on silica gel by medium pressure column chromatography (MPCC). The oily nonpolar fraction I eluted with dichloromethane contained mainly fats, fatty acids and the *peri*-hydroxyquinones, fraction II (CH₂Cl₂/10% MeOH) afforded mainly the faint yellow, strongly blue fluorescent

chartreusin¹). Further separation of the first fraction by PTLC yielded tetrangulol³, fujianmycin A⁴, ochromycinone⁵, desoxyrabelomycin⁵, emycin A⁵, 7-deoxyauramycinone⁶ (**1a**), the resomycins A~C (**2~4**) and a second fraction of chartreusin.

Results and Discussion

7-Deoxyauramycinone (**1a**) and the resomycins A~C (**2~4**) were obtained as orange solids giving a yellow fluorescence under UV light on the TLC plate and a violet colouration with sodium hydroxide, typical for the 1,8-dihydroxyanthraquinone chromophore. The molecular weights and the corresponding formulae were determined by ESI and EI mass spectra and by high resolution of the molecular ions to be *m/z* 382.1054 (C₂₁H₁₈O₇), 382.1053 (C₂₁H₁₈O₇), 364.0946 (C₂₁H₁₆O₆), and 362.0791 (C₂₁H₁₄O₆), respectively.

The proton NMR spectrum of 7-deoxyauramycinone (**1a**) showed two signals of chelated hydroxyl groups at δ 12.49 and 12.09, aromatic proton signals of a 1,2,3-trisubstituted benzene system at δ 7.81, 7.67 and 7.29, and a singlet at δ 7.62. In the aliphatic region, it showed two methyl signals at δ 3.75 and 1.42, of which the former could be assigned to a methyl ester signal and the latter to a methyl group attached to a quaternary *sp*³ carbon. Five further signals each of intensity 1 H indicated a ring system due to the coupling pattern.

The ¹³C NMR spectrum showed 21 carbon signals as demanded by the molecular formula. Two carbonyl signals of a quinone at δ 192.9 and 181.6 with a shift difference of $\Delta\delta$ 11.3 indicated that both hydroxyl groups must be at the same side of the chromophore. An ester carbonyl, twelve aromatic and six aliphatic carbons were detected. A search in AntiBase⁷ with these NMR data, the molecular weight or the molecular formula showed that compound **1a** is identical with 7-deoxyauramycinone, which was confirmed by direct comparison of the NMR data with literature values^{8,10}. As the assignment of the NMR data was not published, 2D spectra were measured. 7-Deoxyauramycinone (**1a**) showed the same sign of the optical rotation as given in the literature for (9*R**,10*R**)-7-deoxyauramycinone¹¹ which showed **1a** to possess the same configuration. The equatorial proton H-8 at δ 1.92 (dddd, ²*J*=13.9; ³*J*=6.8, 3.1; ⁴*J*=1.6 Hz) showed a long

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range W coupling with the proton H-10 at δ 3.91 (s br) indicating the latter to be in the equatorial position as well.

Resomycin A (**2**) possessed the same molecular formula, and the ^1H and ^{13}C NMR spectra were very similar to those of **1a** (Table 1). The main differences in the ^1H NMR data were found in the shift of the methoxy and the H-10 methine protons which were closer together in **2** than in **1a**. Further differences were seen in the shift of H-7 and in the splitting pattern of the methylene protons. All these NMR data indicated compound **2** to be a diastereoisomer of **1a**. In contrast to **1a**, the equatorial proton H-8 (δ 1.81) in **2** did

not show a long range W coupling with H-10 at δ 3.88. An inversion at C-10 in **1a** would result in an axial proton H-10 which cannot give a W coupling with the equatorial H-8. Corresponding to force field calculations, an inversion at C-9 in **1a**, however, should not strongly influence the conformation and leave the W coupling untouched. This leads to the relative configuration $9R^*,10S^*$ in **2**. We suggest to name this new natural product as resomycin A.

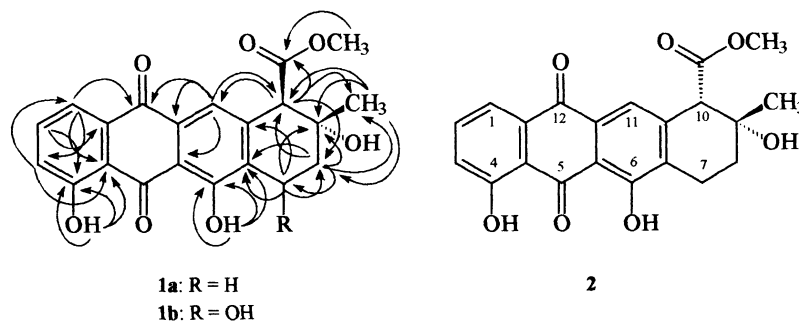
Compound **3** also exhibited similar ^{13}C NMR data as **1a** and **2**, the difference in the molecular weight of 18 pointed to a dehydration product, however. This was supported by

Table 1. ^1H NMR data of 7-deoxyauramycinone (**1a**), resomycin A (**2**), B (**3**) and C (**4**) in deuteriochloroform ($[J]=\text{Hz}$)*.

H No.	1a	2	3	4
1	7.81 (dd, 7.5, 1.1)	7.81 (dd, 7.6, 1.1)	7.82 (dd, 7.5, 1.1)	7.88 (dd, 7.6, 1.1)
2	7.67 (dd, 8.3, 7.5)	7.68 (dd, 8.3, 7.6)	7.66 (dd, 8.3, 7.5)	7.70 (dd, 8.4, 7.6)
3	7.29 (dd, 8.3, 1.1)	7.28 (dd, 8.3, 1.1)	7.28 (dd, 8.3, 1.1)	7.32 (dd, 8.4, 1.1)
7	3.06 (d, 19.3, 6.9, 3.1), 2.87 (ddd, 19.3, 10.2, 6.8)	3.10 (td, 19.4, 6.8), 2.82 (td, 19.4, 6.8)	2.94 (t, 8.4)	8.50 (d, 8.6)
8	2.33 (ddd, 13.9, 10.2, 6.9), 1.92 (dddd, 13.9, 6.8, 3.1, 1.6)	2.32 (td, 13.6, 6.8), 1.81 (td, 13.6, 6.8)	2.42 (t, 8.4)	7.56 (d, 8.6)
9-CH ₃	1.42 (s)	1.34 (s)	2.11 (s)	2.57 (s)
10	3.91 (s br)	3.88 (s)	-	-
11	7.62 (s)	7.59 (s)	7.59 (s)	8.26 (s)
OCH ₃	3.75 (s)	3.87 (s)	3.95 (s)	4.18 (s)
4-OH	12.09 (s)	12.08 (s)	12.10 (s)	12.26 (s)
6-OH	12.49 (s)	12.47 (s)	12.37 (s)	13.76 (s)

* all signal intensities are 1 H and 3 H for the methyl groups, respectively

Fig. 1. HMBC couplings (H \rightarrow C) in 7-deoxyauramycinone (**1a**) and structure of resomycin A (**2**).



the low-field shift of the signals of carbons C-9 and C-10 and of the 3H signal at δ 2.11 of a methyl group attached to a double bond. Correspondingly, the signal of the methine proton H-10 was missing. Careful interpretation of the H,H COSY, HMQC and HMBC correlations unambiguously confirmed structure **3** which we named as resomycin B. The latter (**3**) represents a new natural product, however, the corresponding 4-*O*-methyl derivative was already obtained by synthesis⁸). Comparison of the ¹H NMR data of the latter supports the structure **3** derived by 2D NMR data. Interestingly, the ethanediyl fragment of both compounds delivered in the ¹H NMR spectrum two triplets as for an open chain, and not the pattern expected for a cyclic structure.

The NMR spectra of a fourth component, resomycin C,

were again very similar to those of **3** except that the methylene signals were missing and new proton and carbon signals appeared in the aromatic region. This suggested that resomycin C was fully aromatised. The molecular formula supported this fact, and with the aid of the 2D spectra, structure **4** was confirmed. Resomycin C (**4**) has been discussed as a dehydration product of auramycinone (**1b**)¹²), however, without delivering further details. The isolate GW71/2497 did not produce auramycinone (**1b**) or a similar compound which might act as a precursor of **4** during work-up. The latter is therefore not an artefact but also a natural product. The biological origin of all other isolated quinones is confirmed by their stability during the work-up procedure as well.

Fig. 2. HMBC couplings (\rightarrow) in resomycin B (**3**) and C (**4**).

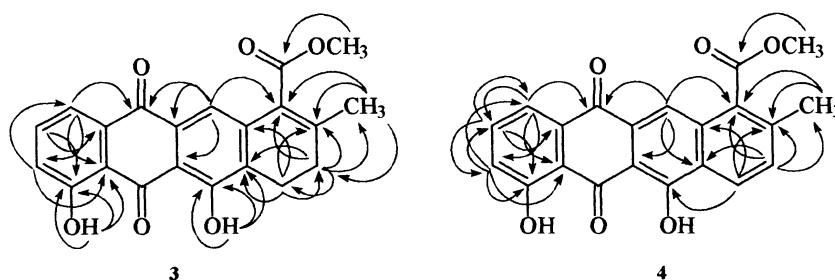


Table 2. ¹³C NMR data of 7-deoxyauramycinone (**1a**), resomycin A (**2**), B (**3**) and C (**4**) in deuteriochloroform.

C No.	1a	2	3	4*	C No.	1a	2	3	4*
1	119.9 (d)	120.4 (d)	120.0 (d)	122.2 (d)	9	69.7 (s)	69.3 (s)	147.9 (s)	142.0 (s)
2	137.1 (d)	137.2 (d)	137.4 (d)	138.2 (d)	10	57.6 (d)	52.7 (d)	127.6 (s)	132.5 (s)
3	124.5 (d)	124.6 (d)	124.7 (d)	126.2 (d)	10a	142.1 (s)	142.1 (s)	140.5 (s)	133.7 (s)
4	162.5 (s)	162.5 (s)	162.8 (s)	162.0 (s)	11	121.2 (d)	119.9 (d)	116.4 (d)	120.5 (d)
4a	116.0 (s)	115.9 (s)	116.4 (s)	117.5 (s)	11a	130.9 (s)	130.8 (s)	130.3 (s)	129.7 (s)
5	192.9 (s)	192.8 (s)	192.9 (s)	192.1 (s)	12	181.6 (s)	181.5 (s)	181.8 (s)	185.6 (s)
5a	113.7 (s)	113.6 (s)	114.6 (s)	109.3 (s)	12a	133.8 (s)	133.7 (s)	134.2 (s)	134.6 (s)
6	161.0 (s)	160.8 (s)	159.6 (s)	164.0 (s)	9-CH ₃	27.5 (q)	27.7 (q)	22.2 (q)	20.7 (q)
6a	133.6 (s)	133.4 (s)	129.4 (s)	127.0 (s)	COCH ₃	171.6 (s)	173.1 (s)	168.5 (s)	173.0 (s)
7	20.2 (t)	21.5 (t)	19.6 (t)	127.6 (d)	COCH ₃	52.5 (q)	56.0 (q)	52.3 (q)	54.7 (q)
8	30.9 (t)	32.3 (t)	29.6 (t)	133.3 (d)	-	-	-	-	-

*measured in CD₂Cl₂/TFA

Table 3. Physico-chemical properties of 1a~4.

	1a	2	3	4
properties	Orange solid	Orange solid	Orange solid	Orange solid
R_f^*	0.46	0.39	0.61	0.56
Molecular formula	$C_{21}H_{18}O_7$	$C_{21}H_{18}O_7$	$C_{21}H_{16}O_6$	$C_{21}H_{14}O_6$
(+)-ESI-MS	385 ([M+H] ⁺ , 27) 787 ([2M+Na] ⁺ , 100)	365 ([M+H] ⁺ , 85) 387 ([M+Na] ⁺ , 100)	365 ([M+H] ⁺ , 85) 387 ([M+Na] ⁺ , 100)	363 ([M+H] ⁺ , 15) 747 ([2M+Na] ⁺ , 100)
(-)-ESI-MS	381 ([M-H] ⁻ , 100), 785 ([2M+Na-2H] ⁻ , 53)	361 ([M-H] ⁻ , 100), 745 ([2M+Na-2H] ⁻ , 16)	363 ([M-H] ⁻ , 100), 749 ([2M+Na-2H] ⁻ , 40), 1113 ([3M+Na- 2H] ⁻ , 80)	361 ([M-H] ⁻ , 100), 745 ([2M+Na-2H] ⁻ , 16)
EI-MS (70 eV)	382 (M ⁺ , 24), 364 (16), 350 (10), 340 (8), 305 (100), 279 (25), 265 (4), 43 (5)	382 (M ⁺ , 27), 364 (14), 350 (11), 340 (8), 305 (100), 279 (29), 265 (4), 43 (5)	364 (M ⁺ , 49), 332 (12), 305 (100), 284 (4), 256 (9), 147 (15), 104 (22), 57 (63)	362 (M ⁺ , 100), 347 (13), 332 (26), 305 (36), 256 (27), 185 (6), 129 (17), 97 (19), 59 (69)
IR (KBr) ν (cm ⁻¹)	3430, 2939, 2925, 1721, 1668, 1621, 1462, 1434, 1415, 1382, 1287, 1243, 1214, 1184, 1159, 1111, 1041, 837, 758, 730	3445, 2938, 2925, 1735, 1668, 1620, 1454, 1414, 1381, 1297, 1244, 1204, 1158, 1126, 1032, 839, 785, 748, 728	3429, 2925, 2820, 1725, 1671, 1636, 1627, 1595, 1474, 1412, 1384, 1270, 1117, 1095, 756, 701	3431, 2988, 2925, 2821, 1726, 1621, 1464, 1458, 1382, 1283, 1264, 1220, 1134, 1027, 983, 765, 707
UV/VIS (CHCl ₃) λ_{max} (lg ϵ):	261 (4.19), 292 (3.79), 435 (3.84)	261 (4.23), 292 (3.83), 435 (3.88)	259 (4.13), 291 (4.28), 445 (3.96)	245 (4.27), 263 (4.36), 292 (4.07), 451 (3.97)
$[\alpha]_D^{20}$ (c 0.1, CHCl ₃)	+67°	-63°	-	-

* Cyclohexane/50 % ethyl acetate

Biological Properties

Antibacterial, antimicrobial and antifungal activities were qualitatively determined using the agar diffusion method. 7-Deoxyauramycinone (1a) and the resomycins A~C (2~4) were active against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus* and *Escherichia coli* with MIC values of ~20 μ g/ml, however inactive against *Mucor miehei* (TÜ 284), *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

Experimental

Materials and methods and antimicrobial tests were used as described earlier¹³. R_f values were measured on Polygram SIL G/UV₂₅₄ (Macherey-Nagel & Co.) with cyclohexane/50% ethyl acetate when not stated otherwise.

Taxonomy of the Producing Strain

The Actinomycete isolate GW71/2497 was obtained from the strain collection of bioLeads in Heidelberg, Germany. It was Gram-positive, aerobic, non-acid fast, and differentiated into substrate and aerial mycelium. The strain

formed aerial hyphae with long hooked or open-coiled chains of spores (*retinaculum-apertum* category). Fragmentation of the substrate mycelium, sporangia or sclerotium-like structures, and flagellated spores were not observed. Aerial hyphae and spore mass were grey on yeast extract-malt agar, oatmeal and soil extract agar. The substrate mycelium was brown on most media. A brown diffusible pigment was formed on yeast extract-malt extract and oatmeal agar. The strain produced melanin pigments on tyrosine agar slants.

Based on the chemotaxonomic properties like presence of L,L-diaminopimelic acid, absence of characteristic sugars in whole cell hydrolysate (chemotype I), growth characteristics, and morphology the strain GW71/2497 most probably belongs to the genus *Streptomyces*. The reference culture of *Streptomyces* sp. isolate GW71/2497 is kept on yeast extract-malt extract agar in the collection of bioLeads company, Heidelberg, Germany.

Soybean Flour-mannitol Medium

Defatted soybean flour (20 g) and mannitol (20 g) were suspended in 1 litre tap water and adjusted to pH 7.8 prior to sterilisation.

Malt Extract - Yeast Extract Medium

Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in 1 litre tap water and adjusted to pH 7.8 prior to sterilisation.

Fermentation of *Streptomyces* sp. Isolate GW71/2497

Ten 1 litre-Erlenmeyer flasks each containing 200 ml of soybean flour/mannitol medium were inoculated with well-grown agar subcultures of *Streptomyces* sp. isolate GW71/2497 and incubated with 95 rpm at 28°C for 3 days. A 20-litre jar fermentor with the same medium was then inoculated with the shaker culture and held at 28°C for 72 hours. The culture broth was mixed with diatom earth (*ca.* 1 kg) and filtered through a pressure filter. The mycelial cake and the culture filtrate were both extracted separately each three times with about 2 litres of ethyl acetate. As the composition of both extracts was similar, the organic phases were combined and concentrated under vacuum at 40°C to get a yellowish brown *semisolid* residue.

On pre-separation of the crude extract by middle pressure column chromatography (MPCC) on silica gel, first the *peri*-hydroxyquinones together with fats and fatty acids were eluted with CH₂Cl₂. Further elution with CH₂Cl₂/10% MeOH delivered chartreusin (>500 mg from fraction II). Successive purification of fraction I by PTLC with dichloromethane/2~6% acetone and then

cyclohexane/30~60% ethyl acetate delivered tetrangulol (5 mg, Rf=0.63), fujianmycin A (30 mg, Rf=0.33), ochromycinone (25 mg, Rf=0.55), desoxyrabelomycin (2 mg, Rf=0.56), emycin A (21 mg, Rf=0.60), 7-deoxyauramycinone (**1a**, 2 mg), and the resomycins A (**2**, 1.5 mg), B (**3**, 4 mg), and C (**4**, 7 mg). The data of the resomycins are listed in Tables 1 and 2.

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References

- 1) LEACH, B. E.; K. M. CALHOUN, L. E. JOHNSON, C. M. TEETERS & W. G. JACKSON: Chartreusin, a new antibiotic produced by *Streptomyces chartreusis*, a new species. *J. Am. Chem. Soc.* 75: 4011~4012, 1953
- 2) MASKEY, R. P.; K. PUSECKER, M. SPEITLING, P. MONECKE, H. LAATSCH & E. HELMKE: 2'- and 4'-Chartreusin-monoacetates, new natural products with unusual anisotropy effects from the marine isolate *Streptomyces* sp. B 5525. *Z. Naturforsch.* 57b: 823~829, 2002
- 3) KUNSTMANN, M. P. & L. A. MITSCHER: The structural characterization of tetrangomycin and tetrangulol. *J. Org. Chem.* 31: 2920~2925, 1966
- 4) RICKARDS, R. W. & J.-P. WU: Fujianmycins A and B, new benz[a]anthraquinone antibiotics from a *Streptomyces* species. *J. Antibiotics* 38: 513~515, 1985
- 5) GERLITZ, M.; G. UDVARNOKI & R. ROHR: Biosyntheses of novel emycins from the mutant strain *Streptomyces cellulosa* ssp. *griseoincarnatus* 1114-2. *Angew. Chem. Intern. Ed. Engl.* 34: 1617~1621, 1995
- 6) HOSHINO, T.; Y. SETOGUCHI & A. FUJIWARA: Glycosidation of natural and chemically synthesized anthracycline aglycones. *J. Antibiotics* 37: 1469~1472, 1984
HOSHINO, T. & A. FUJIWARA: Characterization of the microbial conversion products of auramycinone by *Streptomyces coeruleorubidus* ATCC 31276. *J. Antibiotics* 36: 1463~1467, 1983
- 7) LAATSCH, H.: AntiBase 2000, A Natural Products Database for Rapid Structure Determination. Chemical Concepts, Weinheim 2000 and annual updates; see Internet <http://www.gwdg.de/~ucoc/laatsch/>
- 8) FUJIWARA, A.; T. HOSHINO, M. TAZOE & M. FUJIWARA: New anthracycline antibiotics, auramycins and sulfurmycins. I. Isolation and characterisation of auramycins A and B, and sulfurmycins A and B. *J. Antibiotics* 35: 164~175, 1982
- 9) UNO, H.; Y. NARUTA & K. MARUYAMA: Synthesis of (±)-Aklavinones. Application of the stereocontrolled "Zipper" bicyclocyclisation reaction. *Tetrahedron* 22: 4725~4741, 1984
- 10) GESSON, J.-P.; J.-C. JACQUESY & B. RENOUX: A general and regiospecific route to tetracyclic alkenes in the 11-

- deoxyanthracyclinone series. Application to the total synthesis of (\pm)-auramycinone. *Tetrahedron* 22: 4743~4750, 1984
- 11) We are using here the numbering system of KROHN, K.; M. KLIMARS; H.-J. KÖHLE & E. EBELING: Synthesis of ζ -pyrromycinone, 7-deoxyauramycinone, and 7-deoxyaklavinone *via* ketoester cyclization. *Tetrahedron* 40: 3677~3694, 1984
- 12) KUNNARI, T. J.; K. P. J. YLIHONKO, K. D. KLIKA, P. I. MÄNTSÄLÄ & J. M. L. HAKALA: Hybrid anthracyclines from a genetically engineered *Streptomyces galilaeus* mutant. *J. Org. Chem.* 65: 2851~2855, 2000
- 13) BIABANI, M. A. F.; M. BAAKE, B. LOVISETTO, H. LAATSCH, E. HELMKE & H. WEYLAND: Anthranilamides: New antimicroalgal active substance from a marine *Streptomyces* sp. *J. Antibiotics* 51: 333~340, 1998