# 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 fermentated in the usual manner in a 20 litre jar fermentor at 28 °C for 72 h. The total culture broth was exhaustively extracted with ethyl acetate, and the yellowish brown *semi*solid residue was preseparated 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 ($CH_2Cl_2$/10 % MeOH) afforded mainly the faint yellow, strongly blue fluorescent chartreusin[1)]. Further separation of the first fraction by PTLC yielded tetrangulol[3)], fujianmycin A[4)], ochromycinone[5)], desoxyrabelomycin[5)], emycin A[5)], 7-deoxyauramycinone[6)] (**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 ($C_{21}H_{18}O_7$), 382.1053 ($C_{21}H_{18}O_7$), 364.0946 ($C_{21}H_{16}O_6$), and 362.0791 ($C_{21}H_{14}O_6$), respectively.

((insert table 1 here))

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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 $^{13}C$ 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 Δδ 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 AntiBase[7] 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. Resomycin A (**1a**) showed the same sign of the optical rotation as given in the literature for (9R*,10R*)-7-deoxyauramycinone[11] which showed **1a** to possess the same configuration. The equatorial proton H-8 at δ 1.92 (dddd, $^2J$ = 13.9; $^3J$ = 6.8, 3.1; $^4J$ = 1.6 Hz) showed a long 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 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.

((insert Formula 1 – 2 here))

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 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 synthesis[8]. 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.

(insert Figure 1 here))

((insert table 2 here))

((insert table 3 here))

Biological Properties

Antibacterial, antimicroalgal 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* (BS), *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus* (SA) and *Escherichia coli* (EC) with MIC values of ~20 µg/ml, however inactive against *Mucor miehei* (TÜ 284), *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

## Experimental

Materials & methods and antimicrobial tests were used as described earlier[13]. $R_f$ values were measured on Polygram SIL G/$UV_{254}$ (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 l 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 l 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 inseeded with the shaker culture and held at

28 °C for 72 h. 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 l 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 *semi*solid 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 $CH_2Cl_2$. Further elution with $CH_2Cl_2$/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, $R_f = 0.63$), fujianmycin A (30 mg, $R_f = 0.33$), ochromycinone (25 mg, $R_f = 0.55$), desoxyrabelomycin (2 mg, $R_f = 0.56$), emycin A (21 mg, $R_f = 0.60$), 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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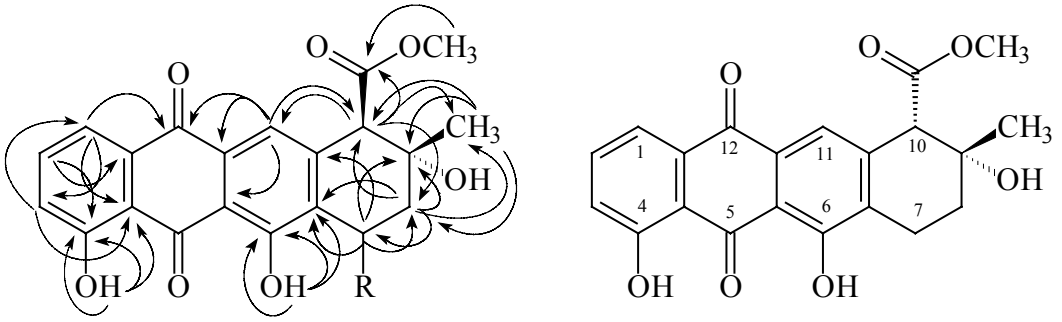
**((Formula 1-2))**

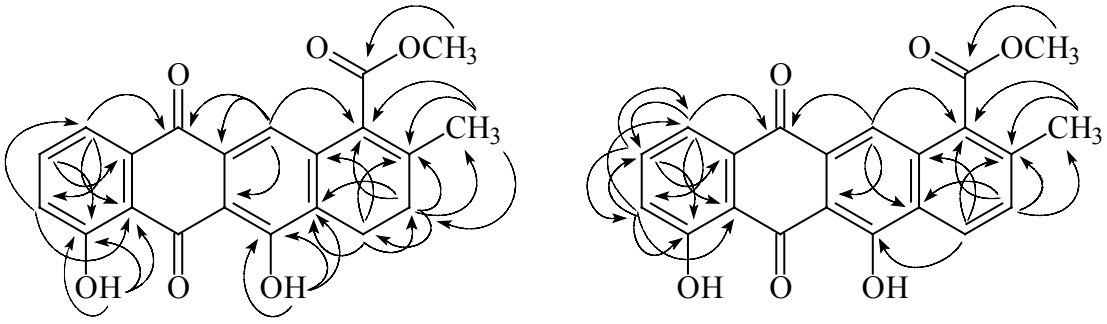



**1a**: R = H
**1b**: R = OH

**2**

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

**((Figure 1))**




**3** **4**

Fig. 3. HMBC couplings (→) in resomycin B (**3**) and C (**4**)

**((Table 1))**

Table 1. $^{1}$H NMR data of 7-deoxyauramycinone (**1a**), resomycin A (**2**), B (**3**) and C (**4**) in deuteriochloroform ([*J*] = 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 (ddd, 19.3, 6.9, 3.1),<br>2.87 (ddd, 19.3, 10.2, 6.8) | 3.10 (td, 19.4, 6.8),<br>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),<br>1.92 (dddd, 13.9, 6.8, 3.1, 1.6) | 2.32 (td, 13.6, 6.8),<br>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

**((table 2))**

Table 2. $^{13}C$ 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) | *C*O$CH_3$ | 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) | CO*C*$H_3$ | 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 $CD_2Cl_2$/TFA

**((Table 3))**

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 ($CHCl_3$) $\lambda_{max}$ (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) |
| $[\alpha]^{20}_D$ (c 0.1, $CHCl_3$) | +67° | -63° | - | - |

* Cyclohexane/50 % ethyl acetate