

# Seitomycin: Isolation, Structure Elucidation and Biological Activity of a New Angucycline Antibiotic from a Terrestrial Streptomyces

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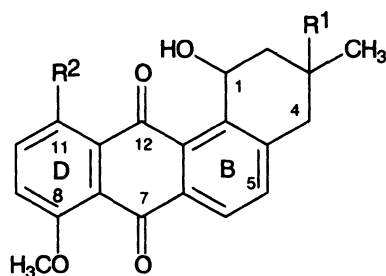
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A new antibiotic, named seitomycin (**1c**), and the known microbial metabolite tetrangulol methyl ether (**2**) were found in the ethyl acetate extract of two terrestrial *Streptomyces* sp. isolates. The structure of the new antibiotic was elucidated by spectroscopic studies and by comparison of the NMR data with the structurally related hatomarubigin C (**1a**) and SM-196 B (**1b**). Seitomycin (**1c**) showed moderate antimicrobial and weak phytotoxic activity, similar to tetrangulol methyl ether (**2**).

*Peri*-hydroxy quinones are very common orange to red coloured microbial metabolites and characterised by the bathochromic shift of their UV absorption in alkaline solution. Among them, the isotetracenones<sup>1–4</sup> constitute a special group of quinone antibiotics with a benz[*a*]anthraquinone chromophore. In the course of our screening program for novel secondary metabolites, we

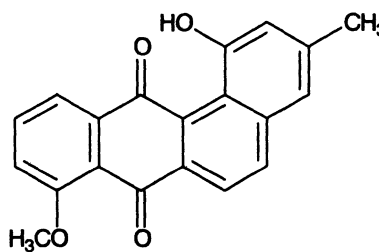
found now a new member of this family designated as seitomycin (**1c**). This paper deals with the isolation, structure elucidation and biological activity of this *peri*-hydroxyquinone antibiotic.



**1a:** R<sup>1</sup> = H, R<sup>2</sup> = OH

**1b:** R<sup>1</sup> = OH, R<sup>2</sup> = H

**1c:** R<sup>1</sup> = OH, R<sup>2</sup> = OH



**2**

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### Taxonomy of the Producing Strain

The *Streptomyces* strain GW19/1251 was obtained from the strain collection of bioLeads in Heidelberg, Germany. This organism was Gram-positive, non-acid fast, grew aerobically, and differentiated into substrate and aerial mycelium. The aerial hyphae were sterile and highly branched. Neither aerial nor substrate mycelium showed fragmentation. The colour of the aerial mycelium was light grey on yeast extract-malt agar and white on oatmeal and soil extract agar. The substrate mycelium was light brown on most media. An orange brown diffusible pigment was formed on yeast extract-malt extract agar and on soil extract agar. Melanin pigments were not produced on tyrosine agar slants. The strain is deposited in the culture collection of the Deutsche Sammlung von Zellkulturen und Mikroorganismen GmbH (Braunschweig, Germany), DSZM number 15463.

### Results and Discussion

The terrestrial *Streptomyces* sp. isolate GW19/1251 was cultured in M<sub>2</sub> medium and worked up using standard conditions<sup>5</sup>. The resulting extract was subjected to column chromatography on Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 3:2). On further separation by PTLC and finally Sephadex LH-20, the fraction containing the orange *peri*-hydroxyquinones delivered two compounds, the known metabolite tetrangulol methyl ether (**2**)<sup>6,7</sup>, and the more

polar seitomycin (**1c**). The same compounds together with feodomycinone C was formed by a second *Streptomyces* sp. GW10/1118 during fermentation on M<sub>2</sub> medium.

Compound **1c** was obtained as an orange solid with the physico-chemical properties summarised in Table 1. The blue violet colour reaction with sodium hydroxide indicated a *peri*-hydroxy quinone, and with anisaldehyde/sulphuric acid it turned first violet and then brown. The molecular weight was determined to be 354 with ESI and EI mass spectroscopy, and the high resolution of the molecular peak resulted in the molecular formula C<sub>20</sub>H<sub>18</sub>O<sub>6</sub>. The IR spectra exhibited signals at 3438 cm<sup>-1</sup> indicating hydroxy groups, and absorptions at 1659 and 1630 cm<sup>-1</sup> were ascribed to non-chelated and chelated quinone carbonyls.

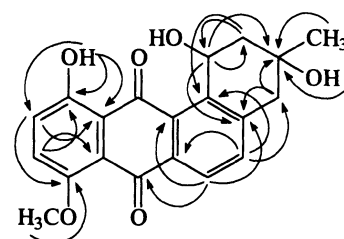
The <sup>1</sup>H NMR spectrum showed the presence of one chelated OH group ( $\delta$  2.63), and two pairs of aromatic protons at  $\delta$  8.23/7.55 and 7.41/7.33 gave *ortho*-coupling doublets. In the aliphatic region, two singlets indicated methoxy and methyl groups, and two AB and ABX signals, respectively, were due to two methylene groups. The <sup>13</sup>C NMR spectrum consisted of 20 signals, of which according to the APT NMR spectrum eleven were due to quaternary carbon atoms including 2 carbonyls at  $\delta$  192.5 and 181.6, five methine, two methylene and two methyl carbons.

In the HMBC spectrum, the first set of *ortho*-positioned aromatic protons at  $\delta$  7.41 and 7.33 was coupling with carbon signals at  $\delta$  157.2 and 119.6, and at  $\delta$  153.8 and 117.2, respectively. The chelated OH signal showed couplings to signals at  $\delta$  157.2, 126.7 and 117.2, and the methoxy protons had correlations with a signal at  $\delta$  153.8. These couplings combined with the H,H COSY and HMQC correlations allowed to construct a *p*-methoxyphenol moiety. Similarly, the other pair of aromatic *ortho*-protons at  $\delta$  8.23 and 7.55, respectively, showed relevant three bond couplings with signals at  $\delta$  181.7, 142.5 and at  $\delta$  130.1, 140.4, 136.1 and 45.9. In addition, the proton signal at  $\delta$  7.55 showed a <sup>2</sup>J coupling with a partner at  $\delta$  142.5 (see Fig. 1). This and the correlation of the

Table 1. Physico-chemical properties of seitomycin (**1c**).

Appearance	orange solid
R <sub>f</sub>	0.57 (CHCl <sub>3</sub> /5 % MeOH).
Molecular formula	C <sub>20</sub> H <sub>18</sub> O <sub>6</sub>
EI MS: <i>m/z</i> (%)	354 (51), 336 (48), 318 (100), 279 (14), 202 (8), 128 (4), 114 (3), 57 (3)
(+)-ESI MS: <i>m/z</i> (%)	731 ([2M+Na <sup>+</sup> ], 24), 399 ([M+2Na-H] <sup>-1</sup> , 100), 355 ([M+H] <sup>+</sup> , 16)
(-)-ESI MS: <i>m/z</i> (%)	353 ([M-H] <sup>-1</sup> , 100)
IR (KBr) $\nu$ cm <sup>-1</sup>	3438, 2962, 2925, 2858, 1659, 1630, 1584, 1469, 1435, 1371, 1258, 1230, 1178, 1103, 1025, 802, 692, 578
UV/VIS (MeOH): $\lambda_{\max}$ (log $\epsilon$ )	259 (3.40), 336 (2.57), 460 (2.80) nm
[ $\alpha$ ] <sub>D</sub> <sup>20</sup>	+132° (c 3.58 mg/ml, MeOH)

Fig. 1. HMBC correlations in seitomycin (**1c**).



aliphatic protons revealed the right half of the molecule. Due to missing couplings of ring D protons with the quinone carbonyls, the position of the methoxy group at C-8 or C-11 could not be deduced from the HMBC spectrum. The  $^{13}\text{C}$  signal of a chelated carbonyl group in a quinone is, however, usually by about  $\Delta\delta$  10 at deeper field as that of the non-chelated carbonyl, whereas in 1,4- and 1,5-dihydroxy-anthraquinones the carbonyl atoms are showing nearly the same shift. Since the 6-H signal couples in the HMBC spectrum with the non-chelated quinone carbonyl at 181.7 (C-7), the methoxy group must be located at C-8 and the phenolic OH group therefore at C-11 in *peri*-position to C-12, resulting in structure **1c** for seitomycin. This is in accordance with the structure of the known tetrangulol methyl ether (**2**) with OMe at the same position which was isolated from *S. sp.* GW19/1251 as well. The structure was

further supported by the similarity of the NMR data with those of hatomarubigin C<sup>4)</sup> (**1a**) and SM-196 B<sup>7)</sup> (**1b**).

#### Biological Activity

Antibacterial and antifungal activities were qualitatively determined using the agar diffusion method with 6 mm paper disc and 5  $\mu\text{g}$  **1c**/disk. Results using *Bacillus subtilis* (BS), *Streptomyces viridochromogenes* Tü 57, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* as test organisms are listed in Table 3. The antibacterial activity of **1c** against these strains was only moderate.

Table 2.  $^{13}\text{C}$  and  $^1\text{H}$  NMR data of seitomycin (**1c**) in  $\text{CDCl}_3$ .

C-No.	$^{13}\text{C}$ NMR	$^1\text{H}$ NMR	C-No.	$^{13}\text{C}$ NMR	$^1\text{H}$ NMR
1	65.4 d	5.64 (t, 6.4 Hz, 1H)	9	123.5 d	7.41 (d, 9.4 Hz, 1H)
2	43.9 t	2.37 (ddd, 13.9, 6.7, 1.5 Hz, 1H), 2.18 (dd, 13.9, 6.1 Hz, 1H)	10	126.7 d	7.33 (d, 9.4 Hz, 1H)
3	69.1 s	-	11	157.2 s	-
4	45.9 t	3.14 (d, 17.3 Hz, 1H), 2.93 (d, 16.9 Hz, 1H)	11a	117.2 s	-
4a	142.5 s	-	12	192.5 s	-
5	136.8 d	7.55 (d, 8.0 Hz, 1H)	12a	130.1 s	-
6	127.4 d	8.23 (d, 7.9 Hz, 1H)	12b	140.4 s	-
6a	136.1 s	-	3-CH <sub>3</sub>	29.4 q	1.52 (s, 3H)
7	181.7 s	-	8-OCH <sub>3</sub>	56.9 q	4.02 (s, 3H)
7a	119.6	-	11-OH	-	12.66 (s, 1H)
8	153.8 s	-	OH	-	4.72 (br s, 1H)

Table 3. Antibacterial activities of **1c** in agar diffusion test with 5  $\mu\text{g}$ /disk (i.d. mm).

	EC	BS	SV	SA	MM	CA	CV	CS	SS
Seitomycin ( <b>1c</b> )	24	29	20	19	0	0	9	8	0
Tetrangulol methyl ether ( <b>2</b> )	18	17	20	17	0	0	0	0	0

BS = *Bacillus subtilis*, SV = *Streptomyces viridochromogenes* (Tü 57), SA = *Staphylococcus aureus*, EC = *Escherichia coli*, CA = *Candida albicans*, MM = *Mucor miehei*, CV = *Chlorella vulgaris*, CS = *Chlorella sorokiniana*, SS = *Scenedesmus subspicatus*.

## Experimental

Material and methods have been described earlier<sup>8)</sup>.

### Fermentation of *Streptomyces* sp. GW19/1251

The terrestrial *Streptomyces* sp. strain GW19/1251 was cultured at 28°C for 3 days on a rotatory shaker (95 rpm) in 80 1 litre-Erlenmeyer each containing 250 ml of M<sub>2</sub> medium consisting of 0.4% glucose, 0.4% yeast extract and 1% malt extract. The pH was adjusted to 7.8 using 2 N NaOH before sterilisation.

The cultured broth from a 20-litre shaker culture was filtered with the aid of diatom earth. The filtrate was extracted four times using ethyl acetate, the mycelial cake was extracted three times with ethyl acetate and then three times with acetone. The acetone extract was concentrated under reduced pressure to give an aqueous residue which was extracted three times with ethyl acetate. Ethyl acetate extracts from both the filtrate and the mycelial cake contained the yellow quinone band of our interest and were combined and concentrated under reduced pressure to deliver 2.57 g of crude extract. The extract was separated on Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 3 : 2) and the biologically active fractions containing the yellow quinone zones were combined. Further purification of the active fraction by PTLC using chloroform/methanol (95 : 5) delivered 6 mg of seitomycin (**1c**) and 4 mg of the less polar tetrangulol methyl ether (**2**). Due to the lack of the biological activity, the rest of the fractions from Sephadex were not further analysed.

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