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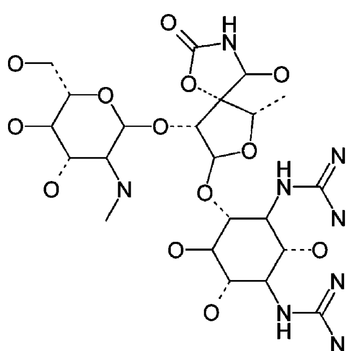
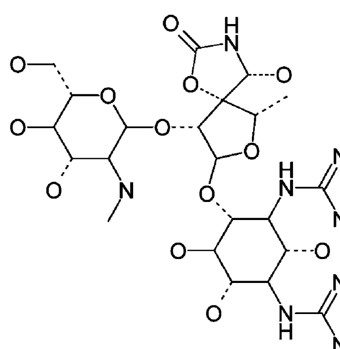


Rhodostreptomycin A



Rhodostreptomycin B

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Rhodostreptomycins, Antibiotics Biosynthesized Following Horizontal Gene Transfer from *Streptomyces padanus* to *Rhodococcus fascians*

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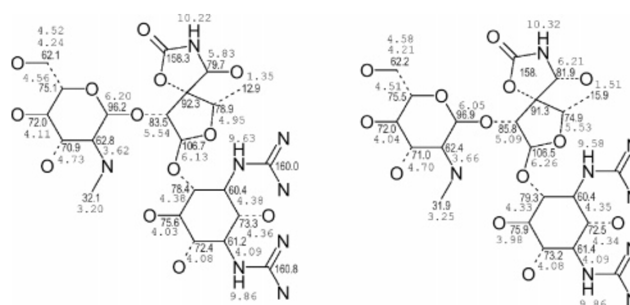
It has become increasingly evident that horizontal gene transfer is central to microbial activities that influence our health and the environment.[1] The influence of horizontal gene transfer in antibiotic production has not been well documented, although there is indirect evidence that it occurs.[2] We carried out competitive co-cultures between a multi-antibiotic resistant mutant of a strain of *Rhodococcus fascians* that does not produce an antibiotic and a strain of *Streptomyces padanus* that is a highly stable actinomycin producer. A strain (307CO) of the *Rhodococcus* emerged from one such culture with the concomitant elimination of the *Streptomyces.* Bioassays of the *Rhodococcus* showed that it produces one or more antibiotics. Genomic analysis revealed that the *Rhodococcus* 307CO harbors a large segment of DNA derived from the *Streptomyces* strain, and there was a correlation between antibiotic production and the presence of the *Streptomyces* DNA in the *Rhodococcus* 307CO.[3] We have isolated two antibiotics, named rhodostreptomycin A (**1**) and B (**2**), from culture broths of *Rhodococcus* 307CO. These appear to be two isomers of a new class of aminoglycosides. In this Communication, we report purification, structure elucidation, and biological activity of the rhodostreptomycins.

Rhodococcus fascians 307CO was cultivated in a production medium consisting of 1% soluble starch, 2% glucose, 2.5% soytone, 0.4% dry yeast, 0.1% beef extract, 0.005% K_2HPO_4 and 0.2% NaCl, pH 7.0, at 27 °C for 5 days. Spent broth exhibited an antimicrobial activity against *S. padanus*. Using assay-guided fractionation, rhodostreptomycins A (**1**) and B (**2**) were purified by a combination of cation exchange (CM-Sephadex) and reversed-phase HPLC (Lichrospher 60RP-select B).

Rhodostreptomycin A (**1**) and B (**2**) were obtained as optically active white powders; $[\alpha]^{20}_D$ −76.19 (*c* 0.042, H_2O), −40.00 (*c* 0.035, H_2O), respectively, that are highly soluble in water, but not in chloroform or *n*-hexane. Physicochemical properties of the compounds are quite similar. Neither isomer exhibited any appreciable UV absorption at wavelengths higher than above 200 nm. Rhodostreptomycins A (**1**) and B (**2**) both had molecular formulas of $C_{22}H_{40}N_8O_{13}$, determined via high-resolution electrospray ionization time-of-flight mass spectrometry. Accurate mass spectra were dominated by signal corresponding to the protonated molecule at m/z = 625.2808 (theoretical m/z = 625.2788).

The hydrocarbon skeleton was revealed by NMR experiments including proton, carbon, GHMBC, DQCOSY, GHMQC, and ROESY or NOESY. The final structures with the correct position of the oxygen and nitrogen heteroatoms were elucidated using tandem ion trapping mass spectrometry. Proton and carbon (boldface) chemical shifts for rhodostreptomycins A (**1**) and B (**2**) are presented below. The two isomers differ in the configuration of the carbon atom bearing the hydroxyl group in the oxazine (C6 in *Strp*), which is *R* in rhodostreptomycin A (**1**) and *S* in rhodostreptomycin B (**2**). Position numbering is based on the individual numbering of the three constituent moieties, namely streptidine (*Strn*), α-L-streptose (*Strp*) and α-L-glucosamine (*Glc*). See Supporting Information.



Rhodostreptomycin A (**1**) Rhodostreptomycin B (**2**)

Analysis of the spectra of rhodostreptomycin A (**1**) started with the methyl signal in the proton spectrum. Couplings in the GHMBC spectrum between the protons and the carbon of this methyl with the proton and carbon at 3.62/62.8 identified position 2 in *Glc*. The sequence of the protons in the *Glc* moiety was revealed by the DQCOSY spectrum. The size of the vicinal proton−proton couplings allowed for the assignment of the relative configuration of the carbons on the *Glc* ring. Coupling between 6.20 and 75.1 confirmed the pyranose ring. The couplings between 6.20 and 83.5 and between 5.54 and 96.2 identified the C−H bound to the oxygen in position 1 of *Glc*. 5.54 couples in the DQCOSY spectrum with 6.13, which couples with the carbons at 92.3 and 78.9. The latter carries 4.95, which couples with 1.35. Couplings of 1.35 with 78.9 and 92.3, together with the chemical shifts, identify the fragment −92.3(O, −) −78.9(4.95, 12.9) −O−. The coupling of 6.13 with 92.3 and 78.9 is possible only when 83.5 is bound to 92.3 and when 106.7 is bound to the oxygen on 78.9. The coupling of 5.54 with 79.7 places the latter on 92.3. The proton on 79.7, 5.83 couples in the DQCOSY spectrum with 10.22, which must be an amide proton, and in the GHMBC spectrum with 78.9, 92.3, and 158.3. The chemical shift of the latter suggests that it is in a urea, carbamate, or guanidine. The product ion fragment at m/z = 144 observed in the MS^3 mass spectrum (Figure 1) indicates that this is a carbamate and confirms the 2-oxo-4-hydroxyoxazine moiety.

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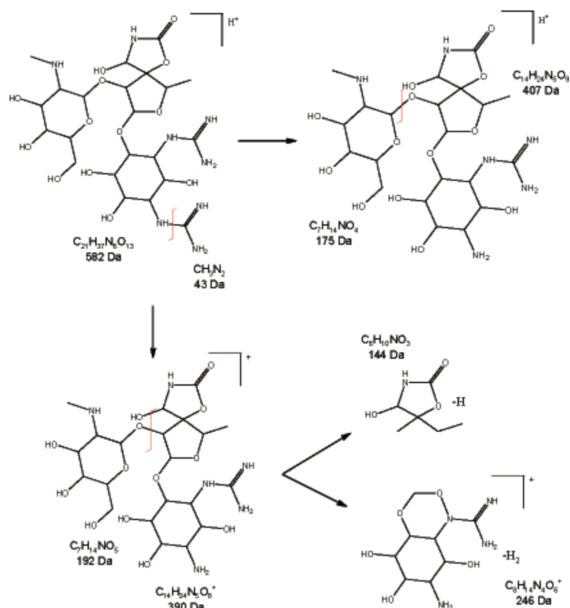

Figure 1. Fragmentation data obtained via tandem mass spectrometry corresponding to the rhodostreptomycin isomers. Identical spectra were obtained for rhodostreptomycins A (**1**) and B (**2**).

The NH vs O in the oxazine ring has to be bound to C6, as indicated by *i*) chemical shifts of C3 and C6 in *Strp* and *ii*) cross-peaks between 5.83 and 10.22 in the DQCOSY and ROESY spectra.

Couplings in the GHMBC spectrum between 6.13/106.7 and 4.38/78.4 identified the latter as position 4 in *Strn*. The guanine protons at 9.63 and 9.86 displayed couplings with the protons at 4.38 and 4.09, respectively. All of the protons on the *Strn* moiety show a triplet with a large coupling constant in the *f2* dimension of the GHMQC spectrum, indicative of their axial positioning.

The NOEs in the ROESY spectrum were used to determine the stereochemistry of the *Strp* moiety. Strong NOEs of 4.95 with 5.54 and 4.38 indicate that 4.95, 5.54 and *Strn* are on the same side of the five-membered ring. The configuration of C6 in *Strp* was elucidated by NOEs between 5.83 and the protons at 4.95 and 1.35. A strong NOE between 5.83 and 4.95 established the configuration of C3 in *Strp*. Other NOEs reveal the conformational preferences about the glycosidic linkages and agree with the conformations found for streptomycin,[4] A/A′ with $\Phi/\Psi_{Strp/Strn}$ 55 ± 10/25 ± 10 and $\Phi/\Psi_{Glc/Strp}$ 55 ± 10/45 ± 10, and B/B′ with $\Phi/\Psi_{Strp/Strn}$ 30 ± 10/−50 ± 10 and $\Phi/\Psi_{Glc/Strp}$ 30 ± 10/−60 ± 10. Representative NOEs are: (i) 4.56 with 6.13 and 5.54; (ii) 3.20 with 1.35; (iii) 9.63 with 5.54; (iv) 4.03 with 5.54; (v) 6.13 with 9.63; (vi) 5.54 with 3.20; (vii) 4.03 with 6.13; (viii) 5.54 with 6.20.

Rhodostreptomycin B (**2**) displays the same couplings in the DQCOSY, GHMQC, and GHMBC spectra as rhodostreptomycin A (**1**). They also yielded the same fragmentation data, indicating identical connectivity. The strong NOE of 6.21 with 5.09 demonstrates that the two isomers have the same stereochemistry at C3 *Strp* and the opposite stereochemistry at C6 *Strp*. Inter-residue NOEs in (**2**) similar to those in (**1**), for example, 4.51 with 5.09 and 6.26, demonstrate that the two compounds exist in similar conformations in pyridine solution.

Table 1. Antimicrobial Activity of Rhodostreptomycins A (**1**) and B (**2**)[a]

organism	diameter of inhibition zone (mm) 1	2
Streptomyces padanus	15	18
Escherichia coli	8	12
Staphylococcus aureus	9	14
Bacillus subtilis	8	14
Helicobacter pylori	10	18
Saccharomyces cerevisiae	0	0

[a] A paper disk (6 mm in diameter) containing each sample (30 μg) was placed on the agar plates.

Rhodostreptomycins exhibited good antibiotic activities against an extensive range of Gram-negative and Gram-positive bacteria, including *H. pylori* in conventional disk assays run on microbial lawns (Table 1). The activity of rhodostreptomycin B (**2**) was more potent than that of rhodostreptomycin A (**1**), suggesting that the difference in stereochemistry between (**1**) and (**2**) influences the biological activity. No evidence of activity against eukaryotes such as *S. cerevisiae* or cytotoxicity against human leukemia (HL-60) was observed.

In conclusion, new isomeric antibiotics produced by *Rhodococcus* 307CO, and consequently named rhodostreptomycin A and B, have been isolated and characterized by mass spectrometry and NMR spectroscopy. Surprisingly, the antibiotics biosynthesized in the *Rhodococcus* following horizontal gene transfer from the *Streptomyces* are aminoglycoside antibiotics and differ widely in the structure from actinomycins, polypeptide antibiotics that are produced by *Streptomyces*.[5] Knowledge concerning rhodostreptomycins production from *Rhodococcus*, especially with regard to the role of gene transfer, could be an attractive research challenge from academic and industrial points of view.

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Supporting Information Available: Experimental details for the fermentation, purification and bioassays; complete NMR data set. This material is available free of charge via the Internet at http://pubs.acs.org.

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