Notes

NEOBERNINAMYCIN, A NEW ANTIBIOTIC PRODUCED BY *MICROCOCCUS LUTEUS*

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In the course of searching for novel compounds that bind to the 50S subunit of the bacterial ribosome and inhibit protein synthesis, we have found a novel antibiotic with potent activity against Gram-positive bacteria and anaerobes. The antibiotic, neoberninamycin, is produced by a strain of *Micrococcus luteus* isolated from a soil sample taken in Highbridge, New Jersey. In this note, we describe the taxonomy of the producing organism, the production, isolation, physico-chemical and biological properties of this novel antibiotic, as well as its relationship to the known sulfur-containing peptide antibiotic, berninamycin A.

Taxonomy

The producing organism is a Gram-positive coccus with the cells arranged in tetrads and irregular clumps of tetrads. The colonies are yellow; the mol % G+C of the DNA is 67. These data place the organism in the genus

Fig. 1. UV spectrum of neoberninamycin.

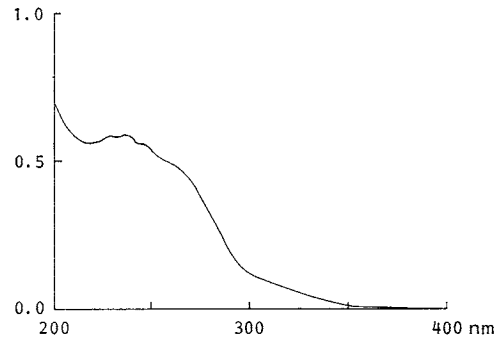
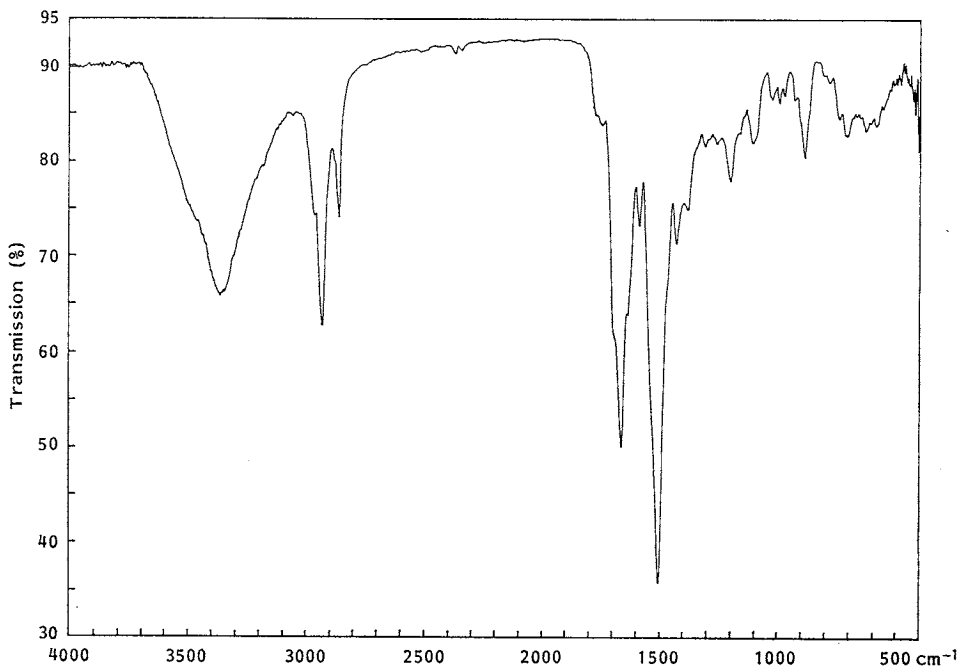


Fig. 2. IR spectrum of neoberninamycin in KBr.

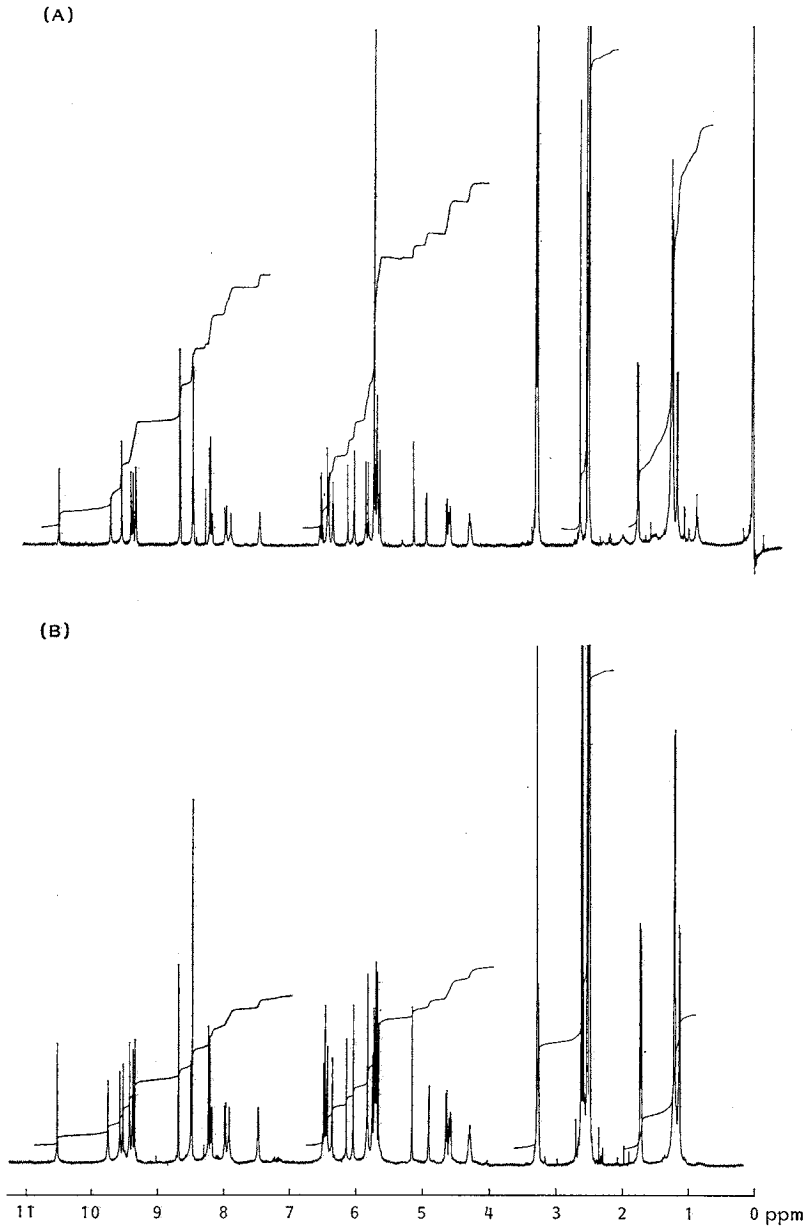


Micrococcus. Further studies of the cultural and physiological characteristics establish it as a strain of *M. luteus*, in accordance with the description given by SCHLEIFER *et al.*[1]. This strain has been deposited in The American Type Culture Collection with the accession No. ATCC 53598.

Production and Isolation

Maximum production of neoberninamycin was obtained after 48 hours incubation at 25°C on a rotary shaker in a medium composed of oatmeal 2% and tomato paste 2%. Production and subsequent isolation steps were monitored by a conventional paper disc-agar diffusion assay with *Staphylococcus aureus*. At harvest, the cultured broth (10 liters) was centrifuged to obtain 700 g of wet cell cake and 9.8 liters of supernatant fluid, both of which contained the antibiotic. The cell cake was extracted with

Fig. 3. 400 MHz ^{1}H NMR comparison of neoberninamycin (A) and berninamycin (B).



MeOH (5 liters) and the extract was concentrated *in vacuo* to an oily residue (50 ml) that was then partitioned between saturated NaCl (250 ml) and CH_2Cl_2 (3×250 ml). The combined organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to give 2 g of an orange oil. The oil was chromatographed on a Whatman LPS-1 silica gel column (2.5×40 cm), eluting with 500 ml portions each of $CHCl_3$, 1% MeOH in $CHCl_3$, 5% MeOH in $CHCl_3$ and 10% MeOH in $CHCl_3$. Neoberninamycin was obtained in the 5% MeOH - $CHCl_3$ eluate which was concentrated *in vacuo* to give 52 mg of crude antibiotic as a yellowish, amorphous solid. Further purification was achieved by preparative reversed-phase HPLC (Whatman M9-50 column containing Partisil ODS-3), eluting with THF - H_2O (35 : 65). Neoberninamycin (22 mg) was obtained as a white amorphous solid. The antibiotic was also isolated from the supernatant fluid by extraction with butanol (2×5 liters). The extract was concentrated *in vacuo* to an oily residue that was partitioned between saturated NaCl and CH_2Cl_2 in the manner described above to yield a brown oil. Purification of the brown oil then proceeded as described for the orange oil obtained from the cell extract.

Physico-chemical Properties and Structural Relationship

The UV spectrum of neoberninamycin exhibits a maximum in CH_3CN at 240 nm ($E^{1\%}_{1cm}$ 360) (Fig. 1) and the IR spectrum (KBr) exhibits major peaks at 3356 (br), 2954, 2924, 2853, 1662, 1636 and 1504 cm^{-1} (Fig. 2). *Anal* found: C 56.87, H 6.56, N 11.14, S 3.3. The fast atom bombardment mass spectra (FAB-MS) have peaks at *m/z* 1,130 in the negative ion mode and 1,132 in the positive ion mode, indicating a nominal MW of 1,131. Since the pseudomolecular ion peaks were not sufficiently intense to allow a high resolution mass measurement and the purity of the sample was not adequate to provide definitive elemental analysis data, a reliable empirical formula is not available. Neoberninamycin is positive to Rydon-Smith reagent and negative to ninhydrin. An acid hydrolysate (6 N HCl), however, is ninhydrin positive. These data indicate that neoberninamycin is a sulfur-containing peptide antibiotic similar to berninamycin A[2] (a correction in the structure of this antibiotic has been recently proposed[3]). Berninamycin A (MW 1,146) gives peaks in the FAB-MS at *m/z* 1,145 in the negative ion mode and at 1,147 in the positive ion mode under the conditions used for neoberninamycin. The ^1H NMR spectra of neoberninamycin and berninamycin A (Fig. 3) while very similar, are clearly distinguishable. In addition to the difference in the mass and ^1H NMR spectra, neoberninamycin and berninamycin A are distinguishable by TLC on silica gel, giving Rf values of 0.23 and 0.27, respectively, with 10% MeOH in $CHCl_3$.

Biological Properties

The results of agar dilution assays against a panel of aerobic and anaerobic bacteria are shown in Table 1. Although inactive against Gram-negative aerobes, neoberninamycin is very active against Gram-positive aerobes and Gram-positive and Gram-negative anaerobes.

Because of the similarity of the physicochemical properties of neoberninamycin and berninamycin A, we investigated the possibility that they have the same mode of action, *i.e.*, inhibition of protein synthesis through an interaction with the 50S ribosomal subunit. THOMPSON *et al.*[4] have provided evidence that berninamycin and thiostrepton have similar mechanisms of action. This conclusion was based, in part, on the observation that neither antibiotic is active against organisms carrying a resistance determinant from *Streptomyces azureus* that catalyzes the *O*-methylation of adenosine in 23S RNA. This methylation occurs at a single, specific site at which the ribo-

Table 1. Activity *in vitro* of neoberninamycin.

Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	0.8
Streptococcus faecalis SC*9011	0.8
Micrococcus luteus SC2495	<0.05
Escherichia coli SC8294	>100
Salmonella typhosa SC1195	>100
Pseudomonas aeruginosa SC8329	>100
Bacteroides fragilis SC10277	1.6
B. thetaiotaomicron SC10278	>100
B. thetaiotaomicron SC9005	0.8
Clostridium perfringens SC11256	<0.05
C. difficile SC11251	50
Fusobacterium necrophorum SC10338	<0.05
Peptostreptococcus anaerobius SC11263	0.1

* SC denotes Squibb Culture collection.

Table 2. Cross-resistance of neoberninamycin, berninamycin A and thiostrepton.

Organism	Inhibition		
	Neoberninamycin	Berninamycin A	Thiostrepton
Streptomyces lividans 1326	+*	+	+
S. lividans 3131 (pIJ702)	−	−	−

* Each compound was tested over a 10-fold concentration range by an agar diffusion assay.

somal protein L11 binds[5]. Such methylated ribosomes do not bind antibiotics that interact in the region of protein L11. Therefore, cross-resistance produced by this determinant suggests antibiotic interaction with this ribosomal site.

Neoberninamycin, berninamycin A and thiostrepton were tested on two strains of *Streptomyces lividans*: A wild type (1326) and a strain (3131) carrying the *S. azureus* resistance determinant (pIJ702). As shown in Table 2, the strain with methylated ribosomes was not inhibited by any of the antibiotics at levels that inhibited growth of the control strain. These cross-resistance data suggest that neoberninamycin acts by the inhibition of protein synthesis through an interaction with the 50S ribosomal subunit. This is not unexpected in light of the physico-chemical similarities between neoberninamycin and berninamycin A.

Thus neoberninamycin, produced by *M. luteus*, is a new antibiotic very closely related to the streptomycete product, berninamycin, but clearly differentiated from it by its mass and ^{1}H NMR spectra.

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