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Verdamicin, a New Broad Spectrum Aminoglycoside Antibiotic

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Verdamicin is a new aminoglycoside antibiotic isolated from fermentation broths of a species of the genus *Micromonospora*, *M. grisea*. It has been differentiated from other known related antibiotics by a variety of chemical and biological methods. Its in vitro and in vivo spectrum of activity appears to be similar to those of gentamicin and sisomicin.

Several species of *Micromonospora*, *M. purpurea*, *M. echinospora*, *M. inyoensis*, and M. spp. 69-683 have previously been described by our laboratory to produce, respectively, the aminoglycoside antibiotics gentamicins, (3), sisomicin, (4), as well as a known streptomycete-produced antibiotic, neomycin (2). In this report we present initial data on a novel aminoglycoside antibiotic named verdamicin, which is produced by a species of *Micromonospora*, *M. grisea*. Preliminary results concerning the producing organism as well as chemical and biological characteristics of verdamicin are presented.

MATERIALS AND METHODS

Producing organism. The organism which produced the antibiotic belongs to the genus *Micromonospora*, *M. grisea*, isolated from soil obtained at Maple Hill Top in Topeka, Kansas. The nomenclature of the species and the name of the antibiotic are derived from the gray-green colony color that is typical of the producing organism on a variety of culture media. A culture of *M. grisea* has been deposited in the collection of the U.S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Ill., where it has been designated as NRRL 3800. Microscopically, the mycelium is long, sparingly branched, and averages about 0.6 μm in diameter. Spores are relatively abundant, born singly on simple sporophores, 1.0 to 1.5 μm in diameter, appearing to be colored brown by transmitted light and to be rough-walled. Detailed taxonomic studies relating to this organism will appear elsewhere.

Fermentation conditions. For laboratory production of verdamicin, a loopful of *M. grisea* from an agar slant or 0.5 ml of a frozen whole preparation was used to inoculate each of a series of 300-ml Erlenmeyer flasks containing 100 ml of the inoculum media (Table 1). The flasks and their contents were incubated for 3 days at 35 C on a rotary shaker. Five milliliters of inoculum were transferred to 500-ml Erlenmeyer flasks containing 100 ml of the fermentation medium (Table 1). The fermentations were carried out at 26 to 35 C for 4 to 7 days with continual agitation on a rotary shaker at 200 to 350 rpm.

Microbiological assay. Antibiotic potencies were determined by means of a disk plate assay with *Staphylococcus aureus* ATCC 6538P as the test organism. The physical conditions of the assay are similar to those described for gentamicin (1). After separation of components as will be described, the verdamicin standard was established. A unit of verdamicin activity is the amount of antibiotic which produces a zonal response of 17.0 ± 1.3 mm under the conditions of the assay and by definition is equal to 1 μg.

Antibiotic isolation. Verdamicin was isolated from an antibiotic mixture found in the fermentation broth by a cation exchange procedure. The pH was adjusted to 2 with mineral acid to release the major portion of the antibiotic from the mycelium. The broth was filtered, neutralized, and adsorbed to an IRC 50 (NH_4^+) ion exchange resin column. The antibiotic mixture was eluted from the resin with 2 N ammonium hydroxide. Separation and isolation of the components was achieved by silica gel chromatography with the lower phase of a solvent mixture consisting of chloroform, isopropanol, and concentrated ammonium hydroxide (2:1:1 vol/vol).

Chromatographic methods. Chromatographic comparisons of a variety of antibiotics were carried out by development on Whatman no. 1 paper in the solvent systems shown in Table 2. The following antibiotics were used in their sulfate forms: verdamicin complex, sisomicin, gentamicin C_1, C_2, C_1a (Schering Corporation, Bloomfield, N.J.), neomycin (Upjohn Co., Kalmazoo, Mich.), kanamycin (Bristol Laboratories, Syracuse, N.Y.), paromomycin (Parke-Davis and Co., Detroit, Mich.), and tobramycin (Eli Lilly and Co., Indianapolis, Ind.).

Hydrolysis of antibiotics. All antibiotics were hydrolyzed for comparative purposes as the free bases, sulfate and *N*-acetyl derivatives (10 mg/ml) in 6 N hydrochloric acid in sealed tubes at 100 C for 2 h.

In vitro studies. For determination of in vitro sensitivity, all test organisms were incubated in Mueller-Hinton broth, pH 7.4, at 37 C for 18 to 24 h except when otherwise indicated. The volume in each tube was 3 ml, and the inoculum was 0.05 ml of a 1:1,000 dilution of an 18-h broth culture.

In vivo studies. Animal studies were carried out in CF-1 male albino mice weighing approximately 20 grams each. Drug solutions were prepared in sterile, distilled water after correction for base content. In

therapeutic tests, animals were treated once by the subcutaneous route, 1 h after intraperitoneal infection with approximately 10^7 organisms per mouse. Control infected mice died in 18 to 24 h; survivors in treated groups were determined 48 h after infection. Generally, groups of seven mice per dosage level (five dose levels), and 10 untreated controls were used for each test. Mean protective dose and mean lethal dose values were determined by probit procedures. The reference antibiotics used were gentamicin sulfate and sisomicin sulfate.

RESULTS AND DISCUSSION

The active fermentation product, consisting of two major components, was compared with a variety of antibiotics by paper chromatography and subsequent bioautography. The R_f values of the two components of the complex were distinguishable from the R_f values of all other related antibiotics, with the exception of sisomicin and gentamicin C_2 (Table 2). The hydrolytic patterns indicated that 2-deoxystreptamine is common to both antibiotics of the complex. However, one of the components was differentiated from other known compounds by R_f and by colors of the hydrolysis products; the hydrolytic pattern of the other components was identical to that of sisomicin. The component of the mixture which was shown to be new was named verdamicin. Further chromatographic and chemical comparisons, as well as IR and NMR spectra, and in vitro and in vivo studies, conclusively demonstrated that the second antibiotic in the mixture was indeed identical to sisomicin, which has been described previously (4). Sisomicin is produced as the major component by *M. inyoensis*.

TABLE 1. *Media for growth of M. grisea for production of verdamicin*

Inoculum medium		Fermentation medium	
Component	Quantity	Component	Quantity
Dextrose	1 g	Dextrose	5 g
Calcium carbonate	2 g	Calcium carbonate	7 g
Beef extract	3 g	Soybean meal	30 g
Tryptose	5 g	Dextrin	50 g
Starch	24 g	Colbalt chloride	10^{-4} to 10^{-6} M
Tap water	1,000 ml	Tap water	1,000 ml

Verdamicin is stable to boiling for at least 30 min in the pH range of 2 to 10. Detailed chemical and physical investigations of verdamacin-free base and sulfate will be described in a separate publication and indicate that verdamicin differs from gentamicin C_2 by the presence of a double bond in one of the amino sugar constituents.

The effects of verdamicin, sisomicin, and gentamicin against some gram-positive organisms are shown in Table 3. Similar activity was obtained with the three antibiotics, except that sisomicin exhibited slightly greater activity than gentamicin or verdamicin against coagulase-positive *S. aureus*.

Data on the activity of the three antibiotics against some gram-negative organisms are summarized in Table 4. Similar activity was noted

TABLE 2. *Comparative R_f values of aminoglycoside antibiotics*

Paper chromatographic system	Verdamicin complex		Sisomicin	Gentamicin components			Neomycin	Kanamycin	Paromomycin
	(1)[a]	(2)[b]		C_1	C_2	C_{1a}			
80% methanol plus 3% sodium chloride (wt/vol) (1:1), descending[c]	0.55	0.45	0.45	0.57	0.56	0.48	0.0, 0.17	0.0, 0.28	0.0, 0.28
Propanol:pyridine:acetic acid:water (6:4:1:3), ascending (V/V)	0.30	0.22	0.22	0.34	0.30	0.22	0.05	0.08	0.07
80% phenol ascending (V/V)	0.45	0.45	0.45	0.45	0.45	0.45	0.0, 0.12	0.0, 0.17	0.0, 0.2
Benzene:methanol (V/V) (9:1), descending	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N-butanol/water:acetic acid (4:5:1) upper phase used ascending	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroform:methanol:17% ammonium hydroxide (2:1:1)[e]	0.40[d]	0.21[d]	0.21[d]	0.67[d]	0.40[d]	0.21[d]			
2-Butanone:*tert*-butanol:methanol: concentrated ammonium hydroxide (16:3:1:6)	0.44[f]	0.36[e]	0.34[f]	0.57[f]	0.47[f]	0.38[f]			

[a] Verdamicin component.
[b] Sisomicin component.
[c] Paper buffered with 0.95 M Na_2SO_4 and 0.05 M $NaHSO_4$.
[d] R_t = distance of zone from origin/distance from origin to end of paper at t = 6 h.
[e] Lower phase.
[f] R_t = distance of zone from origin/distance from origin to end of paper at t = 16 h.

TABLE 3. *Comparative in vitro activity versus gram-positive bacteria*

Organism	No. of strains	Range of MICs (μg/ml)[a]		
		Verdamicin	Gentamicin	Sisomicin
S. aureus				
Coagulase positive	11	0.3-3.0	0.3-3.0	0.008-0.08
Coagulase negative	7	0.08-3.0	0.3-3.0	0.3-3.0
Streptococcus pyogenes	15	0.3-3.0	0.3-7.5	0.3-3.0
Diplococcus pneumoniae	3	3.0	3.0-7.5	0.8-3.0

[a] Mueller-Hinton broth, pH 7.4. MIC, minimal inhibitory concentration.

TABLE 4. *Comparative in vitro activity versus gram-negative bacteria*

Organism	No. of strains	Range of MICs (μg/ml)[a]		
		Verdamicin	Gentamicin	Sisomicin
E. coli	24	0.08-0.3	0.08-0.3	0.08-0.3
E. coli (gentamicin Ad)[b]	2	17.5-37.5	17.5-37.5	17.5-37.5
E. coli (tobramycin-R)[b]	1	0.8	3.0	0.8
Proteus rettgeri	3	0.3	0.3-3.0	0.08-3.0
Proteus morganii	3	0.08-0.3	0.3	0.3
Proteus mirabilis	6	0.3-0.8	0.3	0.08-0.3
Salmonella sp.	6	0.3	0.3-0.8	0.08-0.3
Klebsiella pneumoniae	10	0.03-0.3	0.03-0.3	0.03-0.08
K. pneumoniae (kanamycin-Phos)[b]	1	0.08	0.08	0.03
K. pneumoniae (gentamicin Ad)	1	7.5	7.5	7.5
Serratia sp.	5	0.03-0.3	0.08-0.3	0.03-3.0
Pseudomonas aeruginosa	32	0.8-7.5	0.8-7.5	0.8-3.0
P. aeruginosa (gentamicin-tobramycin-R)	2	>50	>50	>50
P. aeruginosa (gentamicin-Ac)[b]	4	>50	17.5->50	7.5-37.5

[a] Mueller-Hinton broth, pH 7.4.

[b] Abbreviations: Ad, adenylating; R, resistant; Phos, phosphorylating; Ac, acetylating.

for verdamicin, sisomicin, and gentamicin against a group of sensitive *Escherichia coli* strains. All three antibiotics lacked activity against two *E. coli* strains containing the gentamicin adenylylating R-factor, but showed good activity against an *E. coli* strain carrying an R-factor responsible for tobramycin resistance. Similar potencies were observed against a group of *Proteus*, *Salmonella*, and *Klebsiella* strains. None of the antibiotics was very active against a *Klebsiella* strain containing the gentamicin adenylylating R-factor, but all three were highly active against a strain carrying the kanamycin-phosphorylating R-factor. Against several resistant *Pseudomonas* strains, the three antibiotics appeared to be cross-resistant, as evidenced by the lack of activity, or poor activity, against gentamicin-resistant strains and comparable activity against gentamicin-susceptible strains.

The ranges of the relative antibiotic potencies, based on mean protective dose data of verdamicin, gentamicin, sisomicin, are summarized in Table 5. The results are expressed as ratios; e.g., a value of 2.7 for the ratio of verdamicin to gentamicin indicates that on the average, verdamicin was 2.7 times more active than gentamicin against these organisms. These ratios were obtained by determination of the minimal inhibitory concentration value for each antibiotic against each organism and calculation of the difference in activity between antibiotics as a ratio. All of the ratios for a given organism were then averaged to obtain the value shown in the table. These data suggest

TABLE 5. *In vivo activity of verdamicin, gentamicin, and sisomicin in mice*[a]

Organisms	No. of strains	Range (mg/kg)	Verdamicin/gentamicin	Verdamicin/sisomicin	Verdamicin/tobramycin
S. aureus	6	0.2-2.5	2.7[b,c]	1.2[c]	3.0
S. pyogenes	8	2.1-15.3	3.0	1.3	2.3
D. pneumoniae	3	6.2-20.3	2.0	1.6	
E. coli	9	0.3-21.0	1.5	0.9	2.1
K. pneumoniae	7	0.1-3.7	1.5	1.2	1.3
Proteus sp.	6	0.4-7.6	1.7	0.7	0.9
P. aeruginosa	10	1.7-48.0	1.2	0.5	0.7
Salmonella sp.	55	0.3-15.0	3.3	1.6	4.3
Serratia sp.	4	0.4-2.9	1.9	1.3	

[a] Single subcutaneous dose 1 h after infection; mean protective dose.

[b] A value of 2.7 means verdamicin was on an average, 2.7 times more active than gentamicin.

[c] Using gentamicin and sisomicin as reference, but not done in parallel tests.

TABLE 6. *Comparative acute toxicity*

Route[a]	Mean lethal dose (mg/kg)			
	Verdamicin	Gentamicin	Sisomicin	Tobramycin
IV	48	75	34	120
SC	300	480	288	
IP	200	440	221	

[a] IV, intravenous; SC, subcutaneous; and IP, intraperitoneal.

that verdamicin is 2 to 3 times more active than gentamicin or tobramycin against gram-positive organisms, and 1.5 to over 3 times more active against gram-negative organisms, with the exception of *Pseudomonas*. Verdamicin has slightly greater activity against *Pseudomonas* than does gentamicin, and somewhat less activity than does tobramycin. Verdamicin was slightly more active against gram-positive bacteria than sisomicin and was approximately as active as sisomicin against gram-negative bacteria except for *Pseudomonas*. Thus, as noted with in vitro data, verdamicin lies between gentamicin and tobramycin on one hand, and sisomicin on the other, in respect to potency against *E. coli*, *Proteus* sp. and *P. aeruginosa*.

In other studies against a small number of organisms, minimal bactericidal concentration levels were not greatly different than minimal inhibitory concentration (static) levels in common with related aminoglycosides. Initial estimates of serum binding suggest it is low, as is the case with gentamicin and sisomicin.

The comparative acute toxicity of these antibiotics is shown in Table 6. As noted with the protective activity, the acute toxicity of verdamicin in mice more closely relates to that of sisomicin than to either gentamicin or tobramycin.

Verdamicin was shown to be substantially less ataxic in cats than gentamicin, producing ataxia in 26 days, as compared to 16 days for gentamicin at a dosage of 40 mg/kg, 7 days per week.

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LITERATURE CITED

1. Oden, E. M., H. Stander, and M. J. Weinstein. 1964. Microbiological assay of gentamicin, p. 8-13. Antimicrob. Agents Chemother. 1963.
2. Wagman, G. H., J. A. Marquez, P. D. Watkins, J. V. Bailey, F. Gentile, and M. J. Weinstein. 1973. Neomycin production by *Micromonospora* species 69-683. J. Antibiot. **26**:732-736.
3. Weinstein, M. J., G. M. Luedemann, E. M. Oden, and G. H. Wagman. 1964. Gentamicin, a new broad spectrum antibiotic complex, p. 1-7. Antimicrob. Agents Chemother. 1963.
4. Weinstein, M. J., J. A. Marquez, R. T. Testa, G. H. Wagman, E. M. Oden, and J. A. Waitz. 1970. Antibiotic 6640, a new *Micromonospora*-produced aminoglycoside antibiotic. J. Antibiot. **23**:551-554.