Synthesis and Biological Activities of 4"-Deoxy-4"-Sulfonamido-Oleandomycin Derivatives

GENE M. BRIGHT, ARTHUR R. ENGLISH, ARTHUR A. NAGEL, JAMES A. RETSEMA, AND FRANK C. SCIAVOLINO*

Central Research, Pfizer Inc., Groton, Connecticut 06340

Received 11 July 1983/Accepted 10 October 1983

Chemical modification of the macrolide antibiotic oleandomycin (C-1) is described. Reductive amination of 11-acetyl-4"-deoxy-4"-oxo-oleandomycin (C-6) with ammonium acetate provides amino-oleandomycin derivative C-7 in which the 4"-amine is oriented in the axial configuration. The structure-activity relationship of a series of 4"-sulfonamide analogs prepared from amino-oleandomycin derivative C-7 is discussed. Noteworthy is the significant in vitro potency enhancement of the *para*-chlorobenzenesulfonamide analog C-12 over that of the parent oleandomycin. The absolute configuration of the 4"-amino-oleandomycin derivative C-7 was established through X-ray analysis of the *para*-iodobenzenesulfonamide analog C-14.

Oleandomycin (C-1) is a naturally occurring 14-member ring macrolide antibiotic first isolated and characterized in the early 1950s (14). The absolute configuration (Fig. 1) was reported by Celmer in 1965 (3). Oleandomycin possesses a typical macrolide antimicrobial spectrum, demonstrating in vitro activity against a variety of gram-positive organisms, including several strains of erythromycin-resistant staphylococci (6, 7). The in vitro potency of oleandomycin, however, is about one-fourth that of erythromycin against susceptible staphylococcal and streptococcal strains (11, 13). Consequently, a research program was initiated to improve the in vitro potency of oleandomycin through chemical modification. Previous studies concentrated on the preparation of various ester analogs of oleandomycin (2) which improved the pharmacokinetic profile but produced derivatives less potent than the parent antibiotic. Therefore, a new approach was designed, focusing on chemical modification of the oleandrose sugar, since it had previously been shown that the neutral sugar was necessary for maintaining useful antibiotic potency (12).

MATERIALS AND METHODS

Microorganisms. The microorganisms used to assess the spectrum and initial activity were primarily pathogenic strains well adapted to growth under laboratory conditions.

Experimental animals. Male and female mice used in these experiments were purchased from Blue Spruce Farms in Altamont, N.Y. The average weight of the mice used was 13 g.

Minimum inhibitory concentrations. Minimum inhibitory concentrations of the antibiotics were determined by a standard dilution method in brain heart infusion agar for which the multiple inoculator described by Steers et al. was used (10, 15). The standard inoculum contained approximately 20,000 organisms. Incubation was for 18 h at 37°C. The procedure for determining minimum inhibitory concentrations in broth, which utilized an inoculum of about 10⁶ cells per ml, has been described previously (9).

Experimental infections in mice. Acute systemic infections in mice were produced by intraperitoneal inoculation of standardized bacterial cultures suspended in 5% hog gastric

mucin. The challenge dose was generally 1 to 10 lethal doses, i.e., 1 to 10 times the number of organisms needed to kill 100% of the mice within a 4-day period. The dosage regimen for all experimental infections was initiated 0.5 h after challenge, with subsequent doses at 4 and 24 h. After 4 days, a 50% protective dose, expressed in milligrams per kilogram, was calculated by a probit method (1). The 50% protective dose is defined as the dose of antibiotic that will protect 50% of the treated mice from the otherwise lethal infection.

General chemistry methods. Melting points (uncorrected) were determined with a Thomas Hoover capillary apparatus. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian T-60 or XL-100 spectrometer with tetramethylsilane as an internal standard. Only chemical shifts relevant to structural assignment have been reported. Thin-layer chromatography (TLC) was performed with precoated 0.25-mm-thick Silica Gel 60 plates (Merck & Co., Inc., Rahway, N.J.). Vanillin spray, followed by heating, was used to visualize oleandomycin derivatives. Column chromatography used Merck Silica Gel, 70 to 230 mesh.

2'-Acetyl oleandomycin (C-3). To a solution of 10.0 g (14.6 mmol) of oleandomycin (C-1) in 25 ml of methylene chloride was added dropwise 1.41 ml (15 mmol) of acetic anhydride, and the mixture was stirred at room temperature under a nitrogen atmosphere for 3 h. The mixture was added to 50 ml of water, and the pH of the reaction was adjusted to 9.5 with 1 N sodium hydroxide. The organic layer was separated from the aqueous layer, and the aqueous layer was extracted three times with 50 ml of methylene chloride. The organic layers were combined and evaporated to yield 10.0 g (94% yield) of 2'-acetyl oleandomycin (C-3). NMR (δ , CDCl₃), 3.43 (a, 3H), 2.30 (s, 6H), 2.10 (s, 3H); TLC (chloroform-methanol, 9:1), *R_f* = 0.45.

2'-Acetyl-4"-deoxy-4"-oxo-oleandomycin (C-4). To a mixture of 6 ml of benzene and 20 ml of toluene cooled to -5°C under a nitrogen atmosphere was added 467 mg (3.50 mmol) of *N*-chlorosuccinamide. To this turbid solution was added 0.337 ml (5.9 mmol) of dimethyl sulfoxide. After being stirred at 0°C for 20 min, the mixture was cooled to -25°C, and 1.46 g (2.0 mmol) of 2'-acetyl oleandomycin (C-3) in 15 ml of toluene was added. Stirring was continued for 2 h at -20°C, followed by the addition of 0.46 ml (3.3 mmol) of triethylamine. The reaction mixture was maintained at -20°C for an additional 5 min and then was allowed to warm

* Corresponding author.

FIG. 1. Structural features of oleandomycin and analogs.

to 0°C. The mixture was added, with stirring, to 50 ml of water and 50 ml of ethyl acetate. The pH of the aqueous mixture was adjusted to 9.5 by the addition of 1 N sodium hydroxide. The organic layer was separated, dried over sodium sulfate, and concentrated in vacuo to a white foam (1.5 g). Trituration with ether gave 864 mg of crude product, which on recrystallization from methylene chloride-ether gave 212 mg (15% yield) of the pure product (C-4): mp, 183 to 185.5°C; NMR (δ , CDCl₃), 5.60 (1H)m, 3.50 (3H)s, 2.73 (2H)d, 2.23 (6H)s, 2.03 (3H)s. Analysis calculated for C₃₇H₆₁O₁₃N: C, 61.05; H, 8.44; N, 1.92. Found: C, 60.95; H, 8.41; N, 1.98.

2',11-Diacetyl-4"-deoxy-4"-oxo-oleandomycin (C-5). To 4.0 ml (42.4 mmol) of acetic anhydride under a nitrogen atmosphere, cooled to 0°C, was added 727 mg (1.0 mmol) of oleandomycin 4"-ketone, C-4. After 5 min, 0.158 ml (2.0 mmol) of pyridine was added, and the hazy suspension was stirred at room temperature for 16 h. The reaction mixture was added to 100 ml of water and 100 ml of ethyl acetate, and the pH was adjusted to 9.5 with 1 N sodium hydroxide. The

organic layer was separated from the aqueous layer, dried with sodium sulfate, and concentrated under reduced pressure to yield ketone C-5 (588 mg, 73% yield) as an amor-

FIG. 2. Molecular structure of 11-acetyl-4"-deoxy-4"(S)-(para-iodobenzenesulfonamido)-oleandomycin (C-14) as determined by X-ray analysis.

TABLE 1. In vitro activity of oleandomycin and analogs

Compound	Minimum inhibitory concn (µg/ml)								
	<i>Staphylococcus aureus</i>			<i>Streptococcus pyogenes</i>	<i>Streptococcus faecalis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Pasteurella multocida</i>
	01A005	01A052	01A400R	02C203	02A006	51A266	53A009	52A104	59A001
<i>C-1</i>	0.20	0.39	0.39	0.10	0.39	>50	>50	>50	6.25
<i>C-2</i>	0.39	0.78	0.78	0.10	1.56	>50	>50	>50	0.78
<i>C-7</i>	1.56	3.12	1.56	0.78	3.12	25	25	>50	1.56
<i>C-8</i>	1.56	3.12	3.12	0.78	6.25	>50	>50	>50	6.25
<i>C-9</i>	0.20	0.20	0.20	0.10	0.39	50	50	>50	1.56
<i>C-10</i>	0.78	0.78	0.78	0.20	6.25	>50	>50	>50	3.12
<i>C-11</i>	0.20	0.39	0.78	3.12	6.25	50	25	25	0.78
<i>C-12</i>	0.025	0.025	0.025	0.025	0.39	6.25	6.25	25	0.39
<i>C-13</i>	0.10	0.10	0.10	0.05	3.12	25	50	25	1.56
<i>C-14</i>	0.10	0.025	0.10	0.025	0.10	50	25	50	0.39
<i>C-15</i>	0.20	0.20	0.20	0.20	0.20	25	50	50	1.56
<i>C-16</i>	0.20	0.20	0.39	0.05	1.56	25	25	12.5	0.10
<i>C-17</i>	0.20	0.20	0.39	0.10	3.12	12.5	12.5	>50	0.78
<i>C-18</i>	0.10	0.10	0.10	0.10	3.12	12.5	50	>50	3.12

phous foam. Crystallization from isopropyl ether yielded 500 mg of ketone C-5 as white needles: mp, 96 to 98°C; NMR (δ, CDCl₃), 3.48 (3H)s, 2.63 (2H)d, 2.26 (6H)s, 2.06 (6H)s.

11-Acetyl-4"-deoxy-4"-oxo-oleandomycin (C-6). A solution of 4.0 g (4.9 mmol) of diacetate C-5 in 75 ml of methanol was stirred at room temperature for 24 h. The solution was concentrated under reduced pressure to yield 3.6 g (95% yield) of acetate C-6 as an amorphous foam. Crystallization from ether-hexane gave compound C-6 as a white solid: mp, 112 to 117°C; NMR (δ, CDCl₃), 3.43 (3H)s, 2.60 (2H)d, 2.23 (6H)s, 2.01 (3H)s.

11-Acetyl-4"-deoxy-4"(S)-amino-oleandomycin (C-7). To a suspension of 10 g of 10% palladium-on-charcoal in 100 ml of methanol was added 21.2 g of ammonium acetate, and to the resulting slurry was added 20 g (26 mmol) of ketone C-6 dissolved in 100 ml of methanol. The suspension was shaken at room temperature in a hydrogen atmosphere at an initial pressure of 50 lb/in² for 1.5 h. The catalyst was then filtered, and the filtrate was added with stirring to a mixture of 1,200 ml of water and 500 ml of chloroform. The pH was adjusted from 6.4 to 4.5 (1 N hydrochloric acid), and the organic layer was separated from the aqueous layer. The aqueous layer was mixed with 500 ml of ethyl acetate, and the pH of the mixture was adjusted to 9.5 with 1 N sodium hydroxide. The organic layer was separated from the aqueous layer, dried

with sodium sulfate, and concentrated under reduced pressure to a yellow foam. Crystallization from isopropyl ether gave oleandomycin amine (C-7) (6.85 g, 35% yield) as white needles: mp, 157.5 to 160°C; NMR (δ, CDCl₃), 3.41 (3H)s, 2.70 (2H)d, 2.36 (6H)s, 2.10 (3H)s.

General procedure for the preparation of oleandomycin sulfonamides (C-8 through C-18). To a solution of oleandomycin amine (C-7) (1.0 equivalent) dissolved in methylene chloride (8 ml/mmol) under a nitrogen atmosphere was added triethylamine (1.2 equivalents) and the appropriate sulfonyl chloride (1.1 equivalents). The mixture was stirred at room temperature for 16 h and then was added to an equal volume of water. The pH of the mixture was adjusted to 9.5 with 1 N sodium hydroxide. The organic layer was separated from the aqueous layer, dried over sodium sulfate, and concentrated under reduced pressure. The residue was chromatographed on silica gel, with acetone as the eluant, to afford oleandomycin sulfonamides (C-8 through C-18). Presented below are the physical data used to characterize each new oleandomycin sulfonamide derivative.

11-Acetyl-4"-deoxy-4"(S)-methanesulfonamido-oleandomycin (C-8). NMR (δ, CDCl₃), 3.40 (3H)s, 3.06 (3H)s, 2.61 (2H)d, 2.28 (6H)s, 2.06 (3H)s; TLC (chloroform-methanol, 1:1), *R_f* = 0.63.

11-Acetyl-4"-deoxy-4"(S)-benzenesulfonamido-oleandomycin (C-9). NMR (δ, CDCl₃), 7.3 to 8.2 (5H)m, 3.06 (3H)s, 2.63 (2H)d, 2.30 (6H)s, 2.06 (3H)s; TLC (chloroform-methanol, 1:1), *R_f* = 0.56.

11-Acetyl-4"-deoxy-4"(S)-benzylsulfonamido-oleandomycin (C-10). NMR (δ, CDCl₃), 7.36 (5H)s, 4.33 (2H)s, 3.46 (3H)s, 2.63 (2H)d, 2.30 (6H)s, 2.00 (3H)s; TLC (chloroform-methanol, 1:1), *R_f* = 0.55.

11-Acetyl-4"-deoxy-4"(S)-(para-fluorobenzenesulfonamido)-oleandomycin (C-11). NMR (δ, CDCl₃), 7.0 to 8.4 (4H)m, 3.06 (3H)s, 2.66 (2H)d, 2.31 (6H)s, 2.08 (3H)s; TLC (chloroform-methanol, 1:1), *R_f* = 0.60.

11-Acetyl-4"-deoxy-4"(S)-(para-chlorobenzenesulfonamido)-oleandomycin (C-12). mp (acetone-water), 203 to 205°C; NMR (δ, CDCl₃), 7.3 to 8.2 (4H)q, 3.13 (3H)s, 2.73 (2H)d, 2.36 (6H)s, 2.13 (3H)s. Analysis calculated for C₄₃H₆₇O₁₄N₂SCl: C, 57.16; H, 7.47; N, 3.10; Cl, 3.92. Found: C, 56.98; H, 7.41; N, 3.23; Cl, 4.05.

11-Acetyl-4"-deoxy-4"(S)-(para-bromobenzenesulfonamido)-oleandomycin (C-13). NMR (δ, CDCl₃), 7.50 to 7.93 (4H)m, 3.10 (3H)s, 2.66 (2H)d, 2.33 (6H)s, 2.10 (3H)d; TLC (chloro-

TABLE 2. In vivo activity of oleandomycin and analogs against infection by *S. aureus* 01A005 in mice

Compound	50% protective dose (mg/kg)	
	Oral	Subcutaneous
C-1	40	4
C-2	28	16
C-7	>100	<3
C-8	>200	38
C-9	56	11
C-10	>200	10
C-11	>200	18
C-12	27.4	3
C-13	>200	>50
C-14	13.5	12.5
C-15	110	35
C-16	>200	50
C-17	43	8.5
C-18	14	<3

form-methanol, 1:1), $R_f = 0.52$.

11-Acetyl-4"-deoxy-4"(S)-(para-iodobenzenesulfonamido)-oleandomycin (C-14). mp (ethanol-water), 220 to 222°C; NMR (δ , CDCl_3), 7.5 to 8.0 (4H)m, 3.08 (3H)s, 2.66 (2H)d, 2.34 (6H)s, 2.10 (3H)s. Analysis calculated for $\text{C}_{43}\text{H}_{67}\text{O}_{14}\text{N}_2\text{SI}$: C, 51.91; H, 6.79; N, 2.82; S, 3.22. Found: C, 51.35; H, 6.64; N, 2.71; S, 3.28.

11-Acetyl-4"-deoxy-4"(S)-(meta-chlorobenzenesulfonamido)-oleandomycin (C-15). NMR (δ , CDCl_3), 7.3 to 8.0 (4H)m, 3.10 (3H)s, 2.66 (2H)d, 2.33 (6H)s, 2.03 (3H)s; TLC (chloroform-methanol, 1:1), $R_f = 0.45$.

11-Acetyl-4"-deoxy-4"(S)-(3,4-dichlorobenzenesulfonamido)-oleandomycin (C-16). NMR (δ , CDCl_3), 7.2 to 8.1 (3H)m, 3.06 (3H)s, 2.60 (2H)d, 2.30 (6H)s, 2.00 (3H)s; TLC (chloroform-methanol, 1:3), $R_f = 0.31$.

11-Acetyl-4"-deoxy-4"(S)-(2-thienylsulfonamido)-oleandomycin (C-17). mp (isopropyl alcohol), 134 to 135°C; NMR (δ , CDCl_3), 6.8 to 7.8 (3H)m, 3.16 (3H)s, 2.63 (2H)d, 2.30 (6H)s, 2.03 (3H)s. Analysis calculated for $\text{C}_{41}\text{H}_{66}\text{O}_{14}\text{N}_2\text{S}_2 \cdot \text{C}_3\text{H}_7\text{OH}$: C, 56.33; H, 7.76; N, 3.15. Found: C, 56.61; H, 7.60; N, 3.25.

11-Acetyl-4"-deoxy-4"(S)-(3-methyl-2-thienylsulfonamido)-oleandomycin (C-18). mp (ethanol), 184 to 186°C; NMR (δ , CDCl_3), 6.8 to 7.5 (3H)m, 2.96 (3H)s, 2.60 (2H)d, 2.36 (9H)m, 2.00 (3H)s. Analysis calculated for $\text{C}_{42}\text{H}_{68}\text{O}_{14}\text{N}_2$: C, 56.75; H, 7.76; N, 3.15. Found: C, 56.61; H, 7.60; N, 3.25.

RESULTS AND DISCUSSION

Transformation of oleandomycin (C-1) to the 11-acetyl-4"-deoxy-4"-amino-oleandomycin derivative C-7 was identified as the initial chemical objective. The 11-acetate functionality was included in the modification since previous structure-activity relationship studies indicated that acylation of the 11-alcohol improves the pharmacokinetic profile of oleandomycin (7). Therefore, the following chemical sequence was developed.

Acylation of the 2'-hydroxyl group on the desosamine sugar in oleandomycin (C-1), in the absence of an external base, produces 2'-acetyl oleandomycin (C-3) in essentially quantitative yield (4). Presumably, the reaction involves neighboring group participation of the adjacent *N,N*-dimethylamino group and proceeds via an acyl ammonium ion intermediate. Oxidation (5) of the 4"-alcohol yields ketone C-4, which is followed by acylation of the 11-alcohol to yield diacetyl ketone C-5. The overall yield from oleandomycin to ketone C-5 is 47%. Removal of the 2'-acetate from ketone C-5 is accomplished simply by stirring C-5 in methanol at room temperature for 24 h. Reductive amination of resulting ketone C-6 with ammonium acetate in methanol (hydrogen atmosphere, 50 lb/in²; 10% palladium-on-carbon catalyst) produces the 4"-amine derivative C-7 in 60% yield.

The interesting reaction within this synthetic sequence involves the reductive amination of oleandomycin ketone C-5 with ammonium acetate. Presumably, reduction of the intermediate imine can produce either (or both) of the axial and equatorial amine isomers, depending on the approach of the hydrogen source. In this reaction, only the axial amine C-7 is isolated, and less than 10% of the corresponding equatorial isomer can be detected. The stereochemistry of amine C-7 was rigorously proved by X-ray analysis of the iodobenzenesulfonamide derivative C-14 (determined by Jon Bordner, State University of North Carolina at Raleigh) (Fig. 2). Thus, this synthetic transformation converts the original 4"(R)-alcohol absolute configuration in the L-oleandrose sugar to the 4"(S)-amine.

The in vitro and in vivo data for 11-acetyl-4"-deoxy-4"(S)-amino-oleandomycin (C-7) are presented in Tables 1 and 2. It is noteworthy that amine C-7 is less potent than both oleandomycin (C-1) and 11-acetyloleandomycin (C-2) against susceptible *Staphylococcus* and *Streptococcus* isolates. Also, the in vivo oral efficacy of amine C-7 in mice against an infection by *Staphylococcus aureus* 01A005 is less than that of the parent antibiotic. In an effort to increase both the gram-positive potency and the oral efficacy of amine C-7, a series of sulfonamide analogs was synthesized.

Conversion of amine C-7 to the corresponding sulfonamide derivatives C-8 through C-18 by utilizing the appropriate sulfonyl chloride was straightforward. Within the sulfonamide series, the aromatic sulfonamides demonstrated the best potency; alkyl sulfonamide C-8 and benzyl sulfonamide C-10 were less active than phenyl sulfonamide C-9. Incorporation of a halogen onto the aromatic ring led to greater in vitro potency, and in the case of *para*-chlorobenzenesulfonamide C-12, excellent in vivo efficacy was observed. Placement of the chlorine substituent in the *meta* position of the aromatic ring (compound C-15) or incorporation of a second chlorine substituent (compound C-16) failed to enhance the in vitro potency and diminished the in vivo efficacy. Two heteroaromatic sulfonamides, C-17 and C-18, exhibited good oral in vivo efficacy. From this series of compounds, oleandomycin sulfonamides C-12, C-17, and C-18 were selected for further biological investigation, the results of which are reported in the accompanying communication (8).

ACKNOWLEDGMENTS

The authors thank James Clifford, Mark Guadiana, Ernest Grant, Lawrence Vincent, and Lynn Whitaker for expert technical assistance in the preparation of the compounds described in this study.

LITERATURE CITED

1. Batoon, H. E. 1957. An introduction to statistics in the medical sciences, p. 64-67. Burgess Publishing Co., Minneapolis.
2. Celmer, W. D. 1958-1959. Triacetyloleandomycin: biochemical correlations. *Antibiot. Annu.* 1958-1959:277-283.
3. Celmer, W. D. 1965. Macrolide stereochemistry. I. The total absolute configuration of oleandomycin. *J. Am. Chem. Soc.* 87:1797-1799.
4. Celmer, W. D., H. Els, and K. Murai. 1957-1958. Oleandomycin derivatives. Preparation and characterization. *Antibiot. Annu.* 1957-1958:476-483.
5. Corey, E. J., and C. U. Kim. 1972. A new and highly effective method for the oxidation of primary and secondary alcohols to carbonyl compounds. *J. Am. Chem. Soc.* 94:7586-7587.
6. English, A. R. 1957-1958. Oleandomycin: cross-resistance studies with clinical isolates of *Micrococcus pyogenes* var. *aureus*. *Antibiot. Annu.* 1957-1958:756-759.
7. English, A. R., and T. J. McBride. 1959. Laboratory evaluation of partially acetylated esters of oleandomycin. *Proc. Soc. Exp. Biol. Med.* 100:880-884.
8. English, A. R., J. A. Retsema, A. E. Girard, W. Schelkly, and J. E. Lynch. 1984. Evaluation of three 4"-deoxy-4"-sulfonamido-oleandomycin derivatives with erythromycin-like antibacterial potency. *Antimicrob. Agents Chemother.* 25:118-122.
9. English, A. R., J. A. Retsema, V. A. Ray, and J. E. Lynch. 1972. Carbenicillin indanyl sodium, an orally active derivative of carbenicillin. *Antimicrob. Agents Chemother.* 1:185-191.
10. Ericsson, H. M., and J. C. Sherris. 1971. Antibiotic sensitivity testing—report of international collaborative study. *Acta Pathol. Microbiol. Scand. Sect. B* 217(Suppl.):64-68.
11. Jones, W. F., and M. Finland. 1957. Susceptibility of enterococci and of hemolytic streptococci of groups A, B, C, and G to five new antibiotics *in vitro*. *Am. J. Clin. Pathol.* 27:528-533.

12. **Pestka, S., and R. A. Lemahieu.** 1974. Effect of erythromycin analogues on binding of [14 C]erythromycin to *Escherichia coli* ribosomes. *Antimicrob. Agents Chemother.* **6**:479-488.
13. **Semenitz, E.** 1978. The antibacterial activity of oleandomycin and erythromycin—a comparative investigation using microcalorimetry and MIC determination. *J. Antimicrob. Chemother.* **4**:455-457.
14. **Sobin, B., A. R. English, and W. D. Celmer.** 1954-1955. P.A. 105, a new antibiotic. *Antibiot. Annu.* **1954-1955**:827-830.
15. **Steers, E. E., E. L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of antibiotic susceptibility to antibiotics. *Antibiot. Chemother.* **9**:307-311.