Laboratory Evaluation of Partially-Acetylated Esters of Oleandomycin. (24812)

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In vitro and *in vivo* biological evaluation of triacetyloleandomycin,* the fully acetylated derivative of the antibiotic oleandomycin,† has been reported(1). During extensive examination of this new antimicrobial agent, papergram-bioautograph investigation of chromatograms made from sera and urine samples taken from normal volunteers who had ingested triacetyloleandomycin, led to characterization and identification of 6 different biologically active compounds(2). This *in vivo* conversion is considered unique, since it has not been reported for any other antibiotic. This paper documents *in vitro* and *in vivo* evaluation of these various acetylated esters of oleandomycin, studied as independent chemical entities.

Materials and methods. Triacetyloleandomycin, as well as its hydrolytic conversion esters, 2,3-diacetyloleandomycin; 1,3-diacetyloleandomycin; and 3-monoacetyloleandomycin, were utilized as crystalline preparations. The 3 remaining esters, 1,2-diacetyloleandomycin; 2-monoacetyloleandomycin; and 1-monoacetyloleandomycin, were used as homogenous amorphous preparations. The chemical properties and preparation of these acetyl esters of oleandomycin have been reported by Celmer *et al.*(3). *In vitro* sensitivity studies were done in brain heart infusion broth (Difco) utilizing the common 2-fold serial dilution technic. All bacterial cultures used are routinely maintained by daily transfers. Normal, or antibiotic sensitive, microorganisms were employed as well as antibiotic resistant strains, all recent clinical isolates. Experimental infections in mice were produced by intraperitoneal inoculation of standardized cultures(4). Again, both antibiotic sensitive and antibiotic resistant clinical strains of *Staphylococcus aureus* were studied. Therapy was accomplished by oral administration of the experimental compound one half hour after infection. As reported in evaluation of triacetyloleandomycin, dosage range was from 200 by doubling dilutions to 12.5 mg/kg. All results have been calculated, and are presented as the Effective Dose 50% (total weight of antibiotic which protected 50% of mice) and include 19/20 confidence limits(5). Forty to 80 mice were used at each dosage level, depending upon supply of each experimental sample. Pure bred beagles were used as experimental animals in serum level determinations. In each instance, 10 mg/kg of material was administered orally. A small sample of blood was removed at 0, 1, 3, 5, and 7 hours by jugular venipuncture. Immediately after the drawing, blood was allowed to clot at refrigerator temperature. Then serum was removed and assayed by a plate microbiological method with *Sarcina lutea* as the test microorganism. All results indicating biological activity in serum are expressed as oleandomycin base activity.

Results. The anti-microbial activity of oleandomycin, triacetyloleandomycin, and the various partially-acetylated esters is presented in Table I. As indicated earlier, triacetyloleandomycin undergoes a process of de-acetylation *in vivo* to a number of diacetyl and monoacetyl esters. Such a process does not occur *in vitro* in usual bacteriological media (1). Triacetyloleandomycin is approximately 4 to 8 times less active *in vitro* than the parent antibiotic, oleandomycin (Table I). In this Table it is clearly indicated that all the partially-acetylated esters have biological activity against a large number of Gram positive microorganisms, including clinical antibiotic resistant staphylococci, as well as selected Gram negative bacteria. Since *in vitro* activity of each ester varies from microorganism to microorganism, it would be difficult

* Trade name of J. B. Roerig and Co. Division, Chas. Pfizer and Co., for triacetyloleandomycin is Tao.

† Trade name of Chas. Pfizer and Co., Inc., for antibiotic oleandomycin is Matromycin.

TABLE I. *In Vitro* Activity.

Microorganism	MIC—μg/ml							
	Triacetyl-oleando-mycin	2,3-Diacetyl-oleando-mycin	1,3-Diacetyl-oleando-mycin	3-Mono-acetyl-oleando-mycin	1,2-Diacetyl-oleando-mycin	2-Mono-acetyl-oleando-mycin	1-Mono-acetyl-oleando-mycin	Oleando-mycin base
Staphylococcus aureus	3.12	6.25	.78	.78	3.12	3.12	1.56	.78
Streptococcus pyogenes	.78	.78 pi	.78	.39	.39	.39	.19	.19
Streptococcus faecalis	6.25	12.5	3.12	6.25	3.12	3.12	3.12	1.56
Diplococcus pneumoniae	6.25	6.25	6.25	6.25	3.12	3.12	3.12	1.56
Erysipelothrix rhusiopathiae		6.25		.78	3.12	3.12	.78	
Corynebacterium diphtheriae	12.5	1.56	3.12		12.5	12.5		6.25
Listeria monocytogenes	12.5	>100	12.5	6.25	6.25	6.25	3.12	3.12
Bacillus subtilis		25	12.5	.78	3.12	6.25	.78	
Clostridium perfringens		6.25	.78	3.12	12.5	12.5	1.56	
Escherichia coli	>100	>100	>100	>100	>100	>100	>100	>100
Neisseria gonorrhoeae	50	>100	25	25	12.5	12.5	50	25
Hemophilus influenzae	>100	25	6.25		25	>100		3.12
Antibiotic resistant strains of *Staphylococcus aureus*, clinical isolates:								
Fl 1	6.25	12.5	1.56	3.12	12.5	6.25	1.56	1.56
Ma 1	12.5	6.25	6.25	3.12	6.25	6.25	3.12	3.12
KO 3	6.25	6.25	1.56	1.56	6.25	3.12	1.56	.78
KO 4	6.25	3.12	1.56	1.56	6.25	6.25	1.56	.78
102	3.12	6.25	1.56	1.56	3.12	6.25	1.56	1.56
103	6.25	6.25	1.56	1.56	6.25	3.12	1.56	1.56
100	6.25	3.12	1.56	.78	3.12	3.12	1.56	1.56
101	6.25	3.12	3.12	1.56	3.12	3.12	1.56	1.56

to assign any definite rank in activity. At least 3 are considerably more active than triacetyloleandomycin (1,3-diacetyloleandomycin; 1-monoacetyloleandomycin; and 3-monoacetyloleandomycin) and are quite similar to oleandomycin. Three appear comparable to triacetyloleandomycin in *in vitro* activity, 2-monoacetyloleandomycin; 2,3-diacetyloleandomycin; and 1,2-diacetyloleandomycin. However, these 3 are less active *in vitro* than oleandomycin. It appears that as de-acetylated esters approach oleandomycin more closely in structure, greater *in vitro* activity is evident. All 6 esters have *in vitro* activity against the 8 clinical isolates of *Staph. aureus*, which are resistant to a number of commercially available antibiotics (Table I). For example, all esters were active against clinical isolate Fl 1, which is resistant *in vitro* to >100 μg/ml of penicillin, streptomycin, tetracycline,‡ oxytetracycline,‡ and chlortetracycline.§ All esters were active *in vitro* against Ma 1, which is resistant to >100 μg/ml of erythromycin,‖ in addition to those antibiotics just mentioned. Activity was demonstrated against strain 101 which is resistant *in vitro* to all the antibiotics listed here, and to >100 μg/ml of chloramphenicol¶ and

‡ Trade name of Chas. Pfizer and Co., for antibiotic tetracycline is Tetracyn, for the antibiotic oxytetracycline, Terramycin.

§ Trade name of American Cyanamid Co., Lederle Laboratories Division, for the antibiotic chlortetracycline is Aureomycin.

‖ Trade name of Eli Lilly and Co. for the antibiotic erythromycin is Ilotycin.

¶ Trade name of Parke, Davis and Co., for the antibiotic chloramphenicol is Chloromycetin.

TABLE II. Activity against Experimental Infections in Mice.

Organism	ED_{50}—19/20 confidence limits—mg/kg							
	Oleandomycin phosphate	Triacetyloleandomycin	1-Monoacetyloleandomycin	2-Monoacetyloleandomycin	3-Monoacetyloleandomycin	1,2-diacetyloleandomycin	1,3-diacetyloleandomycin	2,3-diacetyloleandomycin
Streptococcus pyogenes	28 (31–25)	36 (42–30)	20 (35–15)	18 (25–13)	20 (35–19)	29 (41–21)	29 (41–21)	85 (135–53)
Diplococcus pneumoniae	110 (132–92)	95 (113–80)	100 (140–71)	>200	>200	>200	>200	>200
*Staphylococcus aureus**	47 (52–43)	56 (62–51)	22 (32–15)	20 (27–15)	27 (40–19)	23 (34–18)	110 (132–91)	160 (184–139)
Antibiotic resistant strains of *Staphylococcus aureus*:								
Fl 1†	38 (47–31)	62 (81–48)	33 (41–26)	30 (41–22)	75 (97–58)	35 (47–27)	84 (104–69)	>200
Ma 1‡	68 (95–49)	70 (87–56)	33 (42–26)	52 (73–38)	71 (92–51)	88 (116–67)	>200	>200
101§	60 (75–48)	60 (75–48)	44 (56–35)	72 (108–48)	71 (92–51)	88 (122–63)	>200	>200
100	34 (42–27)	49 (66–36)	62 (80–48)	19 (24–16)	135 (184–95)	35 (54–28)	67 (89–50)	>200
103†	39 (51–30)	36 (47–28)	34 (49–27)	43 (61–30)	48 (60–37)	42 (60–29)	165 (212–127)	>200
102†	65 (81–52)	105 (126–87)	64 (90–45)	53 (77–35)	81 (109–60)	70 (105–47)	>200	>200
KO 4	53 (58–48)	76 (94–61)	44 (56–34)	36 (51–25)	54 (79–37)	46 (66–32)	175 (248–123)	>200

* Antibiotic sensitive.
† Resistant *in vitro* to >100 μg/ml of penicillin, streptomycin, tetracycline, oxytetracycline, and chlortetracycline.
‡ Resistant *in vitro* to >100 μg/ml of erythromycin, in addition to those antibiotics listed previously.
§ Resistant *in vitro* to >100 μg/ml of chloramphenicol and novobiocin in addition to those antibiotics listed previously.

novobiocin.

In vivo studies. *In vivo* activities of the various esters against 10 experimental infections in mice are presented in Table II. It should be remembered that these experimental infections were produced by normal microorganisms (antibiotic sensitive) as well as microorganisms resistant to commercially available antibiotics. Strains 100, 101, 102, and 103 of *Staph. aureus* are recent clinical isolates.

By visual comparison of ED_{50} with its 19/20 confidence limits, 2-monoacetyloleandomycin; 1-monoacetyloleandomycin; and 1,2-diacetyloleandomycin would be considered most active of the esters. These 3 appear to be at least as active as oleandomycin, the parent antibiotic, and seem to be more active than triacetyloleandomycin itself. The ester, 3-monoacetyloleandomycin, has good *in vivo* activity; however, the ED_{50} and confidence limits against *Staph. aureus* 100 are unusually high as compared to other esters. The anomaly is emphasized by *in vitro* activity shown against this strain, *i.e.*, 0.78 μg/ml. The ester 1,3-diacetyloleandomycin, had selected activity against a number of experimental infections, yet had ED_{50}s of >200 mg against others; 2,3-diacetyloleandomycin had activity against the experimental infections produced by *Streptococcus pyogenes* and the normal *Staph. aureus*. Against the remaining 8 experimental infections, ED_{50}s of >200 mg/kg were obtained.

Biological activity, expressed as μg/ml of oleandomycin base, readily appeared within one hour as peak concentrations after oral administration of each of the 6 hydrolytic esters of triacetyloleandomycin at 10 mg/kg to beagle dogs (Table III). Quantitatively, highest serum levels were detected after oral administration of 2-monoacetyloleandomycin; 1-monoacetyloleandomycin; and 2,3-diacetyloleandomycin. These average serum concentrations obtained during the 0-7 hour experi-

TABLE III. Serum Levels in Dog after Oral Administration.

Antibiotic	Avg serum levels (μg/ml) at designated hr 0	1	3	5	7
2-Monoacetyloleandomycin	0	1.678	.809	.132	n.d.*
Ratio		10/10	10/10	4/10	0/10
1,3-Diacetyloleandomycin	0	.871	.416	.068	n.d.
Ratio		20/20	18/19	7/20	0/20
1,2-Diacetyloleandomycin	0	.976	.659	n.d.	n.d.
Ratio		10/10	10/10	0/9	0/9
1-Monoacetyloleandomycin	0	1.427	.671	.128	.002
Ratio		25/25	24/24	10/25	1/25
3-Monoacetyloleandomycin	0	1.081	.494	.093	.007
Ratio		25/25	20/25	8/25	1/25
2,3-Diacetyloleandomycin	0	1.259	.546	.192	.050
Ratio		25/25	21/24	11/24	4/24
Oleandomycin base	0	2.530	.519	.114	.016
Triacetyloleandomycin	0	1.340	.706	.181	.035

* n.d. = not detected.

mental period for these 3 esters were higher or very comparable to those obtained for triacetyloleandomycin and oleandomycin base. The remaining 3 esters produced serum levels quite similar to each other, but slightly below concentrations detected for triacetyloleandomycin and the base.

Serum levels achieved in man after oral administration of single doses of 250 or 500 mg of the esters, and triacetyloleandomycin are presented in Table IV.** It is obvious that at all time intervals, highest serum concentrations were achieved after oral administration of triacetyloleandomycin. Next in rank would be 1,2-diacetyloleandomycin; 2,3-diacetyloleandomycin; and 1,3-diacetyloleandomycin. A third order would be 1-monoacetyloleandomycin; and fourth would be 2-monoacetyloleandomycin and 3-monoacetyloleandomycin. Oral administration of 500 mg of triacetyloleandomycin results in serum levels 2.16 fold; 2.4 fold; and 1.8 fold at 1, 3, and 6 hours over those obtained by a similar dose of oleandomycin base (Table IV). Similarly, increases in favor of 2,3-diacetyloleandomycin over those of the base at same time intervals were 1.3 fold; 1.3 fold; and 1.1 fold.

TABLE IV. Serum Levels in Man.

Antibiotic	Total dosage, mg (orally)	μg/ml in serum at hr 2	3	6
Triacetyloleandomycin	250	.90	.66	.32
1,2-Diacetyloleandomycin	"	.56	.55	.23
1,3- "	"	.54	.40	.23
1-Monoacetyloleandomycin	"	.44	.40	.17
2- "	"	.29	.28	.14
3- "	"	.29	.27	.18
Triacetyloleandomycin	500	1.99	1.80	.95
2,3-Diacetyloleandomycin	"	1.10	1.00	.60
Oleandomycin base	"	.83	.75	.52

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It would be propitious if one could unequivocally relate *in vitro* data, chemotherapeutic experiments in mice, serum levels in the beagle dog and man, to present a straightforward recognition of the efficacy of a therapeutic agent. Unfortunately, not all of these data can be related to each other so directly. For example, although 2-monoacetyloleandomycin rates as high or higher than triacetyloleandomycin or oleandomycin in *in vitro* activity, in chemotherapeutic activity in mice, and in ability to produce serum levels after oral administration to dogs, it failed by approximately one-third to produce serum levels in man attained by triacetyloleandomycin or oleandomycin. Although 1,3-diacetyloleandomycin and 2,3-diacetyloleandomycin were quite active *in vitro* and produced good serum levels in man and dogs, they were without question, the least active against experimental infections in mice. It is obvious therefore, that the direct rela-

tionship desired for such a variety of data is lacking.

However, it is known that after oral administration of triacetyloleandomycin to man, an ever-changing, dynamic series of events occurs that can be detected in sera and urine, *i.e.*, the de-acetylation of triacetyloleandomycin to at least 6 distinct, biologically active esters. Obvious questions as to time of appearance and disappearance of each, quantitative amount of each, possible effects of each single ester acting in the presence of other biologically active esters as well as oleandomycin, await more precise and novel experimental protocols. As these questions are answered, it is hoped that the relationship between these data will become more apparent.

Summary. Triacetyloleandomycin, the fully acetylated derivative of the antibiotic oleandomycin, is hydrolyzed *in vivo* to at least 6 partially-acetylated esters. *In vitro, in vivo,* and serum concentration studies in the beagle dog and man are presented for these biologically active esters of triacetyloleandomycin.

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