sample, mp 100–102°, was prepared by recrystallization from benzene–2-propanol.

3-[2-[Bis(2'-chloroethyl)amino]ethyl]-5,5-pentamethylenehydantoin (17). Compound **12**, 3.0 g (0.01 mol), was added to 25 ml of phosphorus oxychloride.[10] The mixture was heated at 70–90° for 1.5 hr. Phosphorus oxychloride was removed at reduced pressure to yield an oily residue which was treated with 10 ml of concentrated hydrochloric acid. After the initial exothermic reaction subsided, the mixture was heated on steam bath for 10 min and allowed to cool. The mixture was poured into a cold saturated NaOAc solution and the solution was extracted with ethyl acetate. After washing (H_2O), drying (Na_2SO_4), and evaporation, the ethyl acetate extract yielded an oil which solidified upon cooling. Recrystallization from 2-propanol gave 1.67 g (51%) of **17**, mp 125–126°.

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Aromatic Esters of 5-*O*-Desosaminylerythronolide A Oxime

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Several substituted aromatic esters of the C-3 hydroxyl of 5-*O*-desosaminylerythronolide A oxime were prepared. Ribosomal binding studies showed that meta substituents on the aromatic ring gave the most active analogs. The esters described were all inactive in vivo at the maximum level tested.

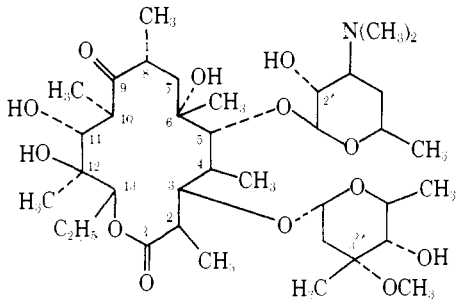
We recently reported the cleavage of the sugar, cladinose, from erythromycin A oxime with 1% HCl in methanol to provide 5-*O*-desosaminylerythronolide A oxime as the corresponding 2'-acetyl acetoxime **1b** after acetylation. The new C-3 hydroxyl of **1b** could be acetylated under vigorous conditions and the C-3 monoacetate was available by selective hydrolysis of the triacetate.[1]

All attempts at glycosylation of the C-3 hydroxyl were unsuccessful. In experiments using acetobromoglucose, decomposition of the reagent was apparently faster than reaction at the C-3 hydroxyl. The glycosylation of 5-*O*-desosaminyldiacetyloleandolide by treatment with methyl L-oleandroside and methanesulfonic acid to give diacetyloleandomycin has been reported.[2] When these conditions were applied to **1b** no incorporation of oleandrose was observed. Treatment of **1b** with glucose tetraacetate and boron trifluoride etherate was unsuccessful due to the instability of **1b** under the reaction conditions. A glycosylation attempt using 3,4,6-tri-*O*-acetyl-1,2-*O*-ethylorthoacetyl-α-D-glucopyranose and mercuric bromide[3] also met with failure. We decided, therefore, to introduce functionality at this hydroxyl via an ester rather than an ether linkage.

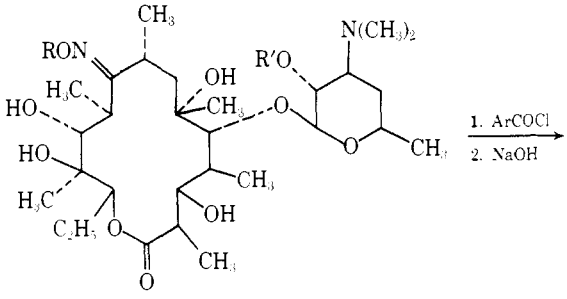
The 3''-methoxyl group of cladinose in erythromycin A is necessary for high activity since erythromycin C with a 3''-hydroxyl group is only 30% as active as erythromycin A.[4] Ribosomal binding of erythromycin A and its analogs can be correlated with antibacterial activity.[5] A hydrogen bond model for this ribosome complex has been proposed.[6] The 3''-methoxyl group is essential for binding probably by a hydrogen bond to a primary amino group of a nucleotide base in the ribosome.[6] We therefore incorporated ring substituents capable of accepting hydrogen bonds in a number of aromatic esters.

The sequence for the preparation of these aromatic esters involved heating **1b** with the appropriate acid chloride in pyridine solution followed by removal of the protecting acetyl groups by base hydrolysis. Aliphatic acid chlorides do not provide the corresponding C-3 esters probably due to competing ketene formation followed by dimerization.

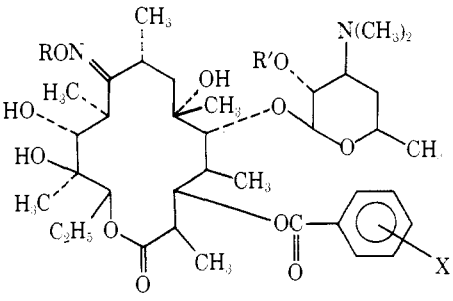
That esterification took place at the C-3 hydroxyl and not at the C-11 hydroxyl was confirmed by regeneration of the 9-ketone by treatment of the *m*-chlorobenzoate **9** with nitrous acid.[1] The resultant ketone exhibited a carbonyl band at 1695 cm^{-1}, the normal position for the erythromycin ketone band. It is reported that esterification of the 11-hydroxyl of erythromycin B causes the ketone absorption to shift to 1705–1708 cm^{-1} because of a decrease in hydrogen bonding.[7]



erythromycin A



1a, R = R′ = H
R = R′ = Ac



	R	R′	X
2	Ac	Ac	H
3	H	H	3-methoxy
4	H	H	4-methoxy
5	H	H	2,3-dimethoxy
6	H	H	3,4,5-trimethoxy
7	H	H	3-nitro
8	H	H	4-nitro
9	H	H	3-chloro
10	H	H	2-fluoro
11	H	H	3-trifluoromethyl

Biological Results. In Vitro Activity. Compounds **5, 7, 9,** and 11 showed 1% of the activity of erythromycin A when tested in an in vitro agar diffusion disk assay against *Staphylococcus aureus* 82 and *Bacillus subtillis* 558. Erythromycin A oxime and erythromycin A are equipotent when assayed against *B. subtilis* 558. The *m*-methoxybenzoate **3** exhibited 2.5% of the erythromycin A activity while the remaining compounds were inactive at 1 mg/ml, the maximum level tested. No activity was observed for any of the compounds against *Escherichia coli* 94, *Pseudomonas aeruginesa* 56, *Saccharomyces cerevisiae* 90, or *Paecilomyces varioti* M-16 at 1 mg/ml.

In Vivo Activity. Mice were infected with 100–1000 MLD's of a diluted overnight broth culture of *Streptococcus pyogenes* or suspensions in 5% gastric mucin of *Proteus vulgaris* and *S. aureus*. Erythromycin A, erythromycin A oxime, and compounds **2, 3,** and **5–10** were administered subcutaneously or orally.[8] Erythromycin A showed its expected activity against *S. pyogenes* (CD_{50} 1.1 mg/kg sc, 19 mg/kg po) and against *S. aureus* (CD_{50} 3.8 mg/kg sc, 16 mg/kg po) but was inactive at 50 mg/kg against the *P. vulgaris* infection. Erythromycin A oxime was less active (CD_{50} 3.0 mg/kg sc, 35 mg/kg po) against *S. pyogenes*. The eight aromatic esters tested were all inactive at 50 mg/kg, the maximum level tested. Compound 4 was not tested because it was not available in sufficient quantity.

Table I

Compd	50% inhibn, μM	Compd	50% inhibn, μM
Erythromycin A	1.3	5	79
Erythromycin A oxime	4	6	83
1a	501	7	60
1b	1770	8	126
2	380	9	59
3	19	10	141
4	269	11	224

Ribosomal Binding. The effect of these and other unrelated erythromycin analogs on the binding of [^{14}C]erythromycin A to *E. coli* ribosomes in a cell-free system has been described elsewhere.[9] These data for the C-3 aromatic esters are now reported in order to demonstrate the relationship between substituents and ribosomal binding. The concentrations of the aromatic esters which produce 50% inhibition of [^{14}C]erythromycin binding to ribosomes are shown in Table I. Although no useful levels of antibacterial activity were found for these esters, it is apparent from the data in the table that large differences in binding activity exist. All of the aromatic esters are more active than the unsubstituted compound 1a and substituents at C-3 of the aromatic ring, especially the 3-methoxy group, provide the most active analogs.

Experimental Section

All melting points were taken in glass capillaries and are corrected. Ir spectra are in $CHCl_3$ solutions, uv spectra are in EtOH solutions, and NMR spectra were determined using a Varian A-60 or HA-100 spectrometer in $CDCl_3$ (Me_4Si). The low-resolution mass spectra were run on a CEC 21-110 instrument at 70 eV by direct insertion. All extracts were dried over anhydrous magnesium sulfate. Analytical and preparative TLC was done on Brinkman silica gel plates using a solvent system of CH_2Cl_2–95% MeOH–NH_4OH (90:10:0.1). The products were eluted from the adsorbent with MeOH. Column chromatography was done on silica gel using the above solvent system.

2′-Acetyl-3-benzoyl-5-*O*-desosaminylerythronolide A Acetoxime (2). To 3.00 g (4.4 mmol) of **1b** in 30 ml of anhydrous pyridine was added 3.0 ml of benzoyl chloride and the reaction mixture was stirred and heated at 90° under an argon atmosphere for 17 hr. The solvent was removed at 0.1 mm and the residue was dissolved in chloroform and washed with 5% sodium bicarbonate solution. After concentration at reduced pressure, a viscous dark oil was obtained. The oil was dissolved in 50 ml of methanol and stirred at room temperature for 24 hr with 8.8 ml of 1 *N* sodium hydroxide. Most of the solvent was removed at reduced pressure and the product was extracted with chloroform. The dried extract was concentrated to 2.89 g of an orange oil. Preparative TLC on silica gel gave 1.44 g of a foam which could not be obtained in crystalline form. Acetylation was accomplished by stirring with 30 ml of acetic anhydride and 0.06 ml of 72% perchloric acid in 300 ml of anhydrous ethyl acetate at room temperature for 1 hr. The solution was washed with saturated sodium bicarbonate solution, dried, stirred for 1 hr with charcoal, and then concentrated at reduced pressure to a gummy solid. Crystallization from ether–hexane gave 1.00 g (29% yield) of **2**: mp 138–148°; ir 1770, 1740, 1735, and 1715 cm^{-1}; NMR δ 2.11 and 2.17 (2 acetyls); λ max 232 nm (ε 13,000). The mass spectrum showed the molecular ion at *m/e* 778. Anal. ($C_{40}H_{62}N_2O_{13}$) C, H, N.

General Procedure. To a solution of 1b in anhydrous pyridine was added the substituted benzoyl chloride and the reaction mixture was stirred and heated at 90° under an argon atmosphere for about 20 hr. After removal of the solvent at 0.1 mm, the residue was dissolved in chloroform and washed with 5% sodium bicarbonate solution. The product obtained on concentration of the chloroform was dissolved in methanol (50 ml/g) and stirred at room temperature for 18 hr with 2 equiv of 1 *N* sodium hydroxide. Most of the solvent was removed at reduced pressure and the product was extracted with chloroform. The dried extract was concentrated at reduced pressure and the crude product was purified by chromatography.

3-(3-Methoxybenzoyl)-5-*O*-desosaminylerythronolide A Oxime (3). A solution of 0.50 g (0.74 mmol) of 1b in 5 ml of anhydrous pyridine and 0.5 ml of *m*-methoxybenzoyl chloride was heated at 90° for 21 hr. Work-up by the general procedure, purification by preparative TLC, and crystallization from ether–hexane gave 0.16 g (30% yield) of 3: mp 149–154°; NMR δ 3.88 (CH_3O–); ir 1730 and 1715 cm^{-1}; λ max 240 nm (ε 8000). The mass spectrum showed the molecular ion at *m/e* 724. Anal. ($C_{37}H_{60}N_2O_{12}$) C, H, N.

3-(4-Methoxybenzoyl)-5-*O*-desosaminylerythronolide A Oxime (4). A solution of 0.50 g (0.74 mmol) of 1b in 5 ml of anhydrous pyridine and 0.5 ml of *p*-methoxybenzoyl chloride was heated at 90° for 24 hr. Work-up by the general procedure, purification by preparative TLC, and crystallization from ether–hexane gave 0.21 g (39% yield) of 4: mp 178–182°; NMR δ 3.84 (CH_3O–); ir 1720 and 1700 cm^{-1}; λ max 260 nm (ε 19,700). The mass spectrum showed the molecular ion at *m/e* 724. Anal. ($C_{37}H_{60}N_2O_{12}$) C, H, N.

3-(2,3-Dimethoxybenzoyl)-5-*O*-desosaminylerythronolide A Oxime (5). To 3.00 g (4.4 mmol) of 1b in 30 ml of anhydrous pyridine was added 2.90 g of 2,3-dimethoxybenzoyl chloride and the solution was heated at 90° for 19 hr. Work-up by the general procedure, column chromatography, and crystallization from methylene chloride–ether gave 0.79 g (24% yield) of 5: mp 221–225°; NMR δ 3.88 and 3.92 (2 methoxyls); ir 1730 and 1710 cm^{-1}; λ max 243 nm (ε 3900). The mass spectrum showed the molecular ion at *m/e* 754. Anal. ($C_{38}H_{62}N_2O_{13}$) C, H, N.

3-(3,4,5-Trimethoxybenzoyl)-5-*O*-desosaminylerythronolide A Oxime (6). To 3.00 g (4.4 mmol) of 1b in 30 ml of anhydrous pyridine was added 3.00 g of 3,4,5-trimethoxybenzoyl chloride and the solution was heated at 90° for 22 hr. Work-up by the general procedure, column chromatography, and crystallization from ether–hexane gave 0.68 g (19% yield) of 6: mp 158–162°; NMR δ 3.89 and 3.91 (3 methoxyls); ir 1730 and 1715 cm^{-1}; λ max 270 nm (ε 7500). The mass spectrum did not give a molecular ion but the highest observed peak was at *m/e* 751 ($M^+ - CH_3 - H_2O$). Anal. ($C_{39}H_{64}N_2O_{14}$) C, H, N.

3-(3-Nitrobenzoyl)-5-*O*-desosaminylerythronolide A Oxime (7). To 3.00 g (4.4 mmol) of 1b in 30 ml of anhydrous pyridine was added 3.00 g of *m*-nitrobenzoyl chloride and the reaction mixture was heated at 90° for 19 hr. Work-up by the general procedure, column chromatography, and crystallization from ether gave 0.91 g (28% yield) of 7: mp 155–160°; ir 1730 and 1720 cm^{-1}; λ max 256 nm (ε 6400). The mass spectrum did not give a molecular ion but the highest observed peak was at *m/e* 706 ($M^+ - CH_3 - H_2O$). Anal. ($C_{36}H_{57}N_3O_{13}$) C, H, N.

3-(4-Nitrobenzoyl)-5-*O*-desosaminylerythronolide A Oxime (8). To 3.00 g (4.4 mmol) of 1b in 30 ml of anhydrous pyridine was added 3.00 g of *p*-nitrobenzoyl chloride and the reaction mixture was heated at 90° for 23 hr. Work-up by the general procedure, column chromatography, and crystallization from ether gave 0.92 g (28% yield) of 8: mp 165–170°; ir 1735 and 1720 cm^{-1}; λ max 260 nm (ε 14,200). The mass spectrum gave a molecular ion at *m/e* 739. Anal. ($C_{36}H_{57}N_3O_{13}$) C, H, N.

3-(3-Chlorobenzoyl)-5-*O*-desosaminylerythronolide A Oxime (9). To 0.50 g (0.74 mmol) of 1b in 5 ml of anhydrous pyridine was added 0.5 ml of *m*-chlorobenzoyl chloride and the reaction mixture was heated at 90° for 24 hr. Work-up by the general procedure, preparative TLC, and crystallization from ether–hexane gave 0.17 g (31% yield) of 9: mp 156–162°; ir 1730 and 1720 cm^{-1}; λ max 233 nm (ε 9200). The mass spectrum gave a molecular ion at *m/e* 728. Anal. ($C_{36}H_{57}ClN_2O_{11}$) C, H, N.

3-(2-Fluorobenzoyl)-5-*O*-desosaminylerythronolide A Oxime (10). To 0.50 g (0.74 mmol) of 1b in 5 ml of anhydrous pyridine was added 0.5 ml of *o*-fluorobenzoyl chloride and the reaction mixture was heated at 90° for 19 hr. Work-up by the general procedure, preparative TLC, and crystallization from ether–hexane gave 0.07 g (13% yield) of 10: mp 150–155°; ir 1750 and 1735 cm^{-1}; λ max 227 nm (ε 12,200). The mass spectrum gave a molecular ion at *m/e* 712. Anal. ($C_{36}H_{57}FN_2O_{11}$) C, H, N.

3-(3-Trifluoromethylbenzoyl)-5-*O*-desosaminylerythronolide A Oxime (11). To 0.50 g (0.74 mmol) of 1b in 5 ml of anhydrous pyridine was added 0.5 ml of *m*-trifluoromethylbenzoyl chloride and the solution was heated at 90° for 19 hr. Work-up by the general procedure, preparative TLC, and crystallization from ether–hexane gave 0.13 g (17% yield) of 11: mp 147–151°; ir 1740 and 1730 cm^{-1}; λ max 228 nm (ε 10,350). The mass spectrum gave a molecular ion at *m/e* 762. Anal. ($C_{37}H_{57}F_3N_2O_{11}$) C, H, N.

3-(3-Chlorobenzoyl)-5-*O*-desosaminylerythronolide A. To 0.295 g (0.4 mmol) of 9 in 30 ml of methanol was added 1.38 g (20 mmol) of sodium nitrite in 9 ml of water. The solution was cooled in an ice bath and 20 ml of 1 *N* hydrochloric acid (20 mmol) was added dropwise over 15 min with stirring. The solution was kept at 3° for 6 hr, solid sodium bicarbonate was added, and then most of the solvent was removed in vacuo. The product was extracted with chloroform and the extract was dried and concentrated. TLC of the product showed some 9 remaining unreacted along with a faster moving spot. Preparative TLC on silica gel and elution of the major band with methanol gave 0.178 g of a foam. Crystallization from ether gave 0.084 g (29% yeild): mp 226–229°; ir 1695 cm^{-1} (>C=O). The molecular ion in the mass spectrum was observed at *m/e* 713. Anal. ($C_{36}H_{56}ClNO_{11}$) C, H, N.

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