Table I. N-Substituted Erythromycylamines 1 (R_1 = H)

R_2	Mp, °C	Analyses	Minimum inhibitory concentration, μg/ml				
			Staph. aureus			*E. coli*	*Proteus morganii*
			6718[a]	U125[b]	12920[c]	AH3	D1
NH_2	125-127		0.5	0.5	128	128	256
$C_6H_5CH_2NH$	181-183	C, H, N, O	0.5	1.0	>512	>512	>512
2,4,6-$Me_3C_6H_2CH_2NH$	158-160	C, H, N, O	0.25	0.25	64	256	256
4-$NO_2C_6H_4CH_2NH$	Amorphous	C, H, N, O	0.25	0.25	64	512	>512
Me_2CHNH	136-139	C, H, N, O	2	2	128	512	512
Cyclohexyl NH	146-150	C, H, N, O	4	4	128	256	512
$NCCH_2CH_2NH$	117-120	C, H, N, O	2	1.0	256	256	512
$C_6H_5COCH_2CH_2NH$	125-128	C, H, N, O	0.25	0.25	64	64	>512
$MeO_2CCH_2CH_2NH$	115-117	C, H, N, O	1.0	2	256	256	>512
$HOCH_2CH_2CH_2NH$	115-118	C, H, N, O	2	2	64	512	>512
$MeCH(OH)CH_2CH_2NH$	136-138	C, H, N, O	0.5	1.0	64	64	256
MeCONH	162-167	C, H, N, O	32	32	>512	> 512	>512
EtCONH	201-202	C, H, N, O	256	256	>512	> 512	>512
C_6H_5CONH	153-160	C, H, N, O	>512	256	>512	> 512	>512
$C_6H_5SO_2NH$	134-135	C, H, N, O, S	4	8	>512	> 512	>512
MeNHCONH	166-170	C, H, N, O	>512	>512	>512	> 512	>512
$C_6H_5NHCONH$	196-200	C, H, N, O	>512	>512	>512	> 512	>512
$C_6H_5NHCSNH$	174-178	C, H, N, O, S	64	32	>512	> 512	>512
EtNHCSNH	148-155	C, H, N, O, S	32	32	>512	> 512	>512
Erythromycin (for comparison)			0.5	0.5	>512	> 512	>512

[a]Penicillin and erythromycin sensitive. [b]Penicillin resistant erythromycin sensitive. [c]Penicillin and erythromycin resistant.

with CH_2Cl_2. The dried ($MgSO_4$) extracts were evaporated to an oil which was crystallized and recrystallized from aqueous EtOH giving 2.6 g (44%), mp 136-139°, pure by tlc. The ir spectrum showed no C=N stretch. *Anal.* ($C_{47}H_{82}N_2O_{12}$) C, H, N.

N-Cyclohexylerythromycylamine was prepared similarly.

N-(2-Benzoylethyl)erythromycylamine. A solution of erythromycylamine (5.0 g, 6.8 mmol) and phenyl vinyl ketone (1.0 g, 7.6 mmol) in MeOH (40 ml) was heated under reflux for 30 min. Tlc indicated complete conversion to a faster running compound. The solvent was evaporated and the resulting oil crystallized and recrystallized from CH_2Cl_2-Et_2O giving 2.7 g (46%), mp 125-128°, pure by tlc: ir (KBr) 1703 cm^{-1} (CO). *Anal.* ($C_{46}H_{78}N_2O_{13}$) C, H, N.

N-(2-Carbomethoxyethyl)- and *N*-(2-cyanoethyl)erythromycylamine were prepared similarly from methyl acrylate and acrylonitrile, respectively.

N-(3-Hydroxybutyl)erythromycylamine. To a solution of erythromycylamine (5.0 g, 6.8 mmol) in MeOH (30 ml) was added methyl vinyl ketone (0.53 g, 7.5 mmol). After 1 hr, tlc showed virtually complete conversion to a faster running compound. Without isolation of this product, sodium borohydride (0.5 g, excess) was added in portions during 90 min. After a further 30 min, the bulk of the solvent was evaporated, water added, and the solution extracted three times with CH_2Cl_2. The dried ($MgSO_4$) extracts were evaporated to an oil which was crystallized and recrystallized from Et_2O giving 2.9 g (53%), mp 136-138°, pure by tlc. *Anal.* ($C_{41}H_{78}N_2O_{13}$) C, H, N.

N-(3-Hydroxypropyl)erythromycylamine was similarly prepared from acrolein.

N-Acetylerythromycylamine. A solution of erythromycylamine (5.0 g, 6.8 mmol) and Ac_2O (0.76 g, 7.5 mmol) in MeOH (30 ml) was kept at room temperature for 10 min. Tlc showed complete conversion to a faster running compound. Et_2O was added and the solution washed with water after adjusting pH to 11 with 2 *N* NaOH. The ether layer was dried ($MgSO_4$) and eventually deposited *N*-acetylerythromycylamine (1.3 g, 25%), mp 162-167°, which was pure by tlc: ir (KBr) 1660, 1520 cm^{-1} (CONH). *Anal.* ($C_{39}H_{72}N_2O_{13}$) C, H, N.

N-Propionyl-, *N*-benzoyl-, and *N*-phenylsulfonylerythromycylamine were prepared similarly using $(EtCO)_2O$, BzCl, and $PhSO_2Cl$, respectively.

N,N'-Erythromycylmethylurea. To a solution of erythromycylamine (5.0 g, 6.8 mmol) in CH_2Cl_2 was added methyl isocyanate (0.5 g, 8.8 mmol). After 10 min tlc showed complete conversion to a faster running compound. Et_2O was added giving crystals (4.0 g) and recrystallized from EtOH-Et_2O giving 3.2 g (59.5%), mp 166-170°, pure by tlc: ir (KBr) 1640 cm^{-1} (urea). *Anal.* ($C_{39}H_{73}N_3O_{13}$) C, H, N.

N,N'-Erythromycylphenylurea, -ethylthiourea, and -phenylthiourea were prepared similarly by reaction with PhNCO, EtNCS, and PhNCS, respectively.

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Antimicrobial Action of Isomeric Fatty Acids on Group A *Streptococcus*

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Previous workers have shown the importance of unsaturation to the germicidal action of fatty acids on gram-positive microorganisms.[1-4] The cis form of the unsaturated long-chain fatty acid (oleic acid) is more active than the saturated compound (stearic acid), and toxicity against organisms increased with increase in the number of double bonds.[5]

While these generalizations are substantially true, little is known concerning the effect that the position and kind of unsaturation has on fatty acid antimicrobial action. Recently, Jenkin, *et al.*, have presented patterns of utilization of isomeric octadecenoic acids in *Leptospira*[6] and in monkey kidney cells.[7] The purpose of the present report was to determine whether generalizations made for *Leptospira* were applicable to another organism, to evaluate the biological activity of the unsaturated dodecyl (short-chain) *vs.* octadecyl (long-chain) series, and to contrast the effectiveness of ethylenic *vs.* acetylenic unsaturation on biological activity.

Experimental Section

Long-chain esters used in this study were synthesized and purified as previously reported.[8,9] Because previous studies[2,5] indicated that the methyl esters of fatty acids were inactive, the synthesized esters were converted to soaps by mild saponification for 24 hr at room temperature. In order to measure any adverse effects or incompleteness of the saponification procedure, methyl laurate, methyl oleate, and linoleic acid were used as control compounds. Methyl esters (10 mg) were dissolved in approximately 0.25 ml of methanol and an equal volume of methanolic 0.5 *M* KOH was added. After standing for 24 hr at room temperature, 10 ml of sterile *Trypticase* soy broth (BBL) was added with vigorous stirring to each tube (pH 8.3). The same broth mixture was used to prepare dilutions of the fatty acid salts. Solutions containing various amounts of lipid were inoculated with 0.05 ml of an 18-hr broth culture (10^6 organisms) of a virulent strain of group A *Streptococcus* (isolated at Providence Hospital, Southfield, Mich. 1970). Lipid concentrations after the second dilution (pH 7.6) had no appreciable effect on the pH of the media.

The minimal inhibitory concentration (MIC) in μmol/ml for each fatty acid was determined after an overnight incubation of the lipid and microorganism at 35° in a 5% CO_2 atmosphere. The MIC was determined by observing the highest dilution of the soap which inhibited growth when compared to an uninoculated chemical control tube.

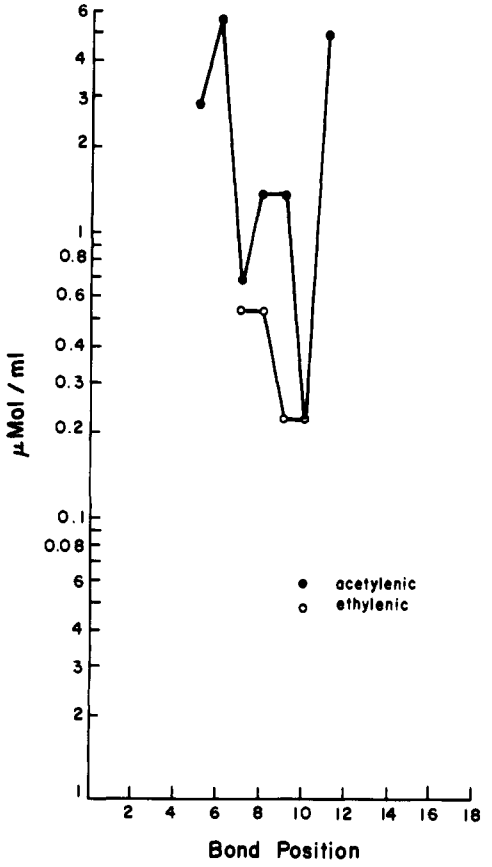

Figure 1. The MIC values for unsaturated lauric acid derivatives are presented. All compounds are less toxic than lauric acid (MIC = 0.12 μmol/ml).

Results

The control methyl esters (12:0 and 18:1) used to check the effect of the saponification procedure indicated complete hydrolysis of the ester since the formed soap and the free acid tested in the same manner had identical MIC values. Linoleic acid also was processed by the same method. No decomposition could be detected since chromatographing the treated and untreated fatty acid resulted in identical thin-layer patterns. Individual fatty acids, other than those used as model compounds, may be more or less susceptible to the saponification procedure. It was not possible because of limited quantities of material available to examine each test fatty acid as in the case of the control compounds. The limitation of test material also prevented us from screening the fatty acids against other microorganisms or other strains. However, in a previous study[10] it has been shown that fatty acids or their derivatives active against one strain retain a similar order of activity against a variety (four to six) of strains of that species. Thus, while the activity measured may have general application, the possibility of resistance among strains is always present. With these qualifications in mind, the following results on 12:1 and 18:1 ethylenic (cis) and acetylenic isomers are presented.

Data for the 12:1 isomers are presented in Figure 1. For the small number of acids compared, the dodecanoic acids were generally more toxic to the group A *Streptococcus* than the corresponding acetylenic isomers. The greatest difference between the two types of unsaturation is found with the Δ^9 isomer. For both types of unsaturation, the Δ^{10} isomer was the most toxic C_{12} acid, *i.e.*, lowest MIC value.

By comparison, the 18:1 series of fatty acid salts were more toxic to group A *Streptococcus* than representatives of the 12:1 family. These data, divided into two homologous series of isomers, odd *vs.* even carbon chain length, are presented in Figure 2. For each series the ethylenic and acetylenic are plotted together. The octadecenoates indicate less toxicity than acetylenic isomers. However, since the data for Δ^6, Δ^7, Δ^8, Δ^{10}, Δ^{12}, Δ^{14}, and Δ^{17} isomers represent only one tube difference in dilution, it is not considered significant. While the differences between ethylenic and acetylenic compounds at positions Δ^4 and Δ^{15} are significant, they are of a reverse order. Consequently, little can be made of the difference in kind of unsaturation present. Peak inhibitory activity for both types of linkages is noted when unsaturation was located in the Δ^2 or Δ^8 positions.

The effect of a second double bond and its positional isomers was examined by studying a number of 18:2 fatty acids. These lipids are more toxic than the 18:1 series. For comparative purposes, individual monoenoic isomers (two) which have unsaturation in similar positions to the dienoic molecule are compared to the dienoic acid; *i.e.*, the $\Delta^{9,12}$ 18:2 is compared to the Δ^9 18:1 and the Δ^{12} 18:1. The differences are listed (Table I). In such a comparison, the activity of the 18:2 is always greater than the component 18:1 isomers. Usually the more unsaturated fatty acids are 8-15-fold more toxic. Contrasted to the results found for the monoenoic fatty acids, the position of the second double bond in the six 18:2 isomers tested did not seem important in terms of enhancing antimicrobial activity.

Discussion

In these experiments we have confirmed an earlier conclusion that the addition of one or two cis double bonds to stearic acid increases the toxicity to various microorgan-

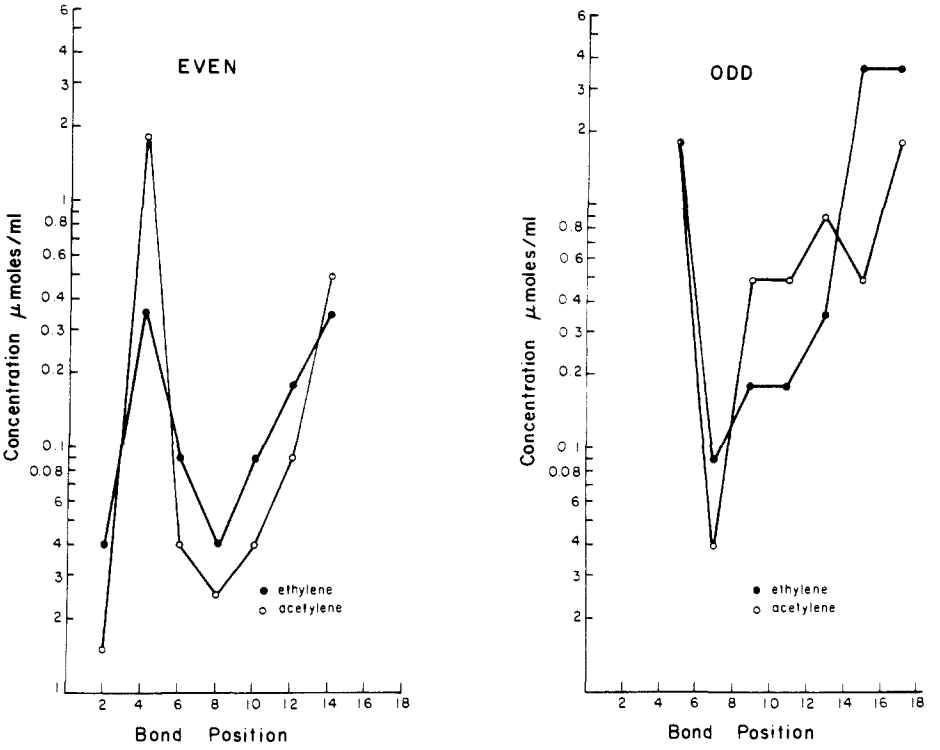

Figure 2. The MIC values for unsaturated C_{18} acids. In general, and opposite to shorter chain fatty acids, these unsaturated acids have lower MIC values than C 18:0 (O > 3.52 μmol/ml).

Table I. Comparison of Ethylenic 18:2 *vs.* 18:1 MIC's against Group A *Streptococcus*

Dienoic	Monoenoic	MIC, μmol/ml	Difference
$\Delta^{9,12}$		0.01	
	Δ^{12}	0.18	0.17
	Δ^{9}	0.18	0.17
$\Delta^{8,12}$		0.02	
	Δ^{12}	0.18	0.16
	Δ^{8}	0.04	0.02
$\Delta^{7,12}$		0.02	
	Δ^{12}	0.18	0.16
	Δ^{7}	0.09	0.07
$\Delta^{6,12}$		0.01	
	Δ^{12}	0.18	0.17
	Δ^{6}	0.09	0.08
$\Delta^{5,12}$		0.02	
	Δ^{12}	0.18	0.16
	Δ^{5}	1.77	1.75
$\Delta^{6,12}$		0.01	
	Δ^{6}	0.09	0.08
	Δ^{12}	0.18	0.17
$\Delta^{6,11}$		0.02	
	Δ^{6}	0.09	0.07
	Δ^{11}	0.18	0.16
$\Delta^{6,10}$		0.01	
	Δ^{6}	0.09	0.08
	Δ^{10}	0.09	0.08

isms.[1-4] Contrary to this accepted generalization for long-chain fatty acids, unsaturation in a lower chain fatty acid does not always lead to greater activity. Lauric acid has a lower MIC (0.12 μmol/ml) than the most inhibitory ethylenic or acetylenic isomer (0.25 μmol/ml). Apparently, a critical structure of property of the fatty acid is reached with *twelve carbon saturated* and *eighteen carbon unsaturated* fatty acids. Compounds with greater (>18:0) or lesser (<12:0) chain length are less effective.[5]

The type of unsaturation is unimportant. Earlier, Baylis[1] indicated that the replacement of an ethylenic bond by an acetylenic linkage caused a marked decrease in germicidal activity. We found that the effectiveness of unsaturation in a fatty acid is a function of chain length and bond position. A specific statement concerning the type of unsaturation cannot be made at this time.

While the type of unsaturation is unimportant, the position of unsaturation is critical. As previously shown by Jenkin, *et al.*,[6] the Δ^2 and Δ^8 18:1 isomers were highly inhibitory to *Leptospira interrogans* serotype *Pactoc.* Our results confirm the importance of these same two isomers on group A *Streptococcus.* In contrast to these toxic effects on bacterial cells, the addition of these same fatty acid isomers to monkey kidney cell caused stimulation of growth.[7] Hence, inhibitory or stimulatory effects seem to be a function of positional isomer and cell type.

One of the more interesting generalizations is made by noting the position of unsaturation on biological activity. Maximum effect was found in the Δ^2, Δ^7, and Δ^8 position of the C_{18} acids while the importance of the Δ^{10} position for both linkages was observed in the C_{12} series.

Eight 18:2 isomers were evaluated for their biological activity against group A *Streptococcus* (Table I). No significant effect on differences in antibacterial activity was evident although all were more active than 18:1 isomers. The present report confirms the notion that a second double bond in a long-chain fatty acid further increases toxicity to a microorganism.[1,11,12] A specific statement relating to the location of the second double bond to antimicrobial action cannot be made.

While the conclusions reached in these particular experiments are limited to the effect of compounds on one genus, they do add to our store of knowledge on the general effect of positional isomers.[6,7] Such information will allow us to better differentiate toxic effects against specific organisms or cell types.

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Quaternary Salts of Substituted 2-Aminoethyl *N*-Benzoylaminobenzoate. A New Class of Smooth Muscle Relaxant Agents

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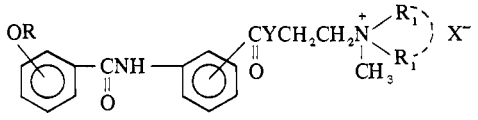
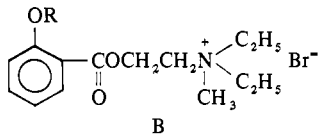
We have described previously[1] the synthesis and smooth muscle relaxant properties of some *N*-(dialkylaminoalkyl) *p*-(2-alkoxybenzamido)benzoate quaternary salts. In order to gain further insight on structure-activity relationships, we have synthesized several other compounds referable to the general formulas A and B.



A



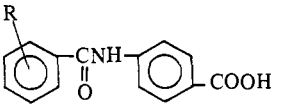
B

Chemistry. The above-mentioned compounds were synthesized from the corresponding bases by quaternization with methyl halides. The bases were obtained by condensation of the appropriate acyl chloride with N-disubstituted 2-aminoethanol (method A) or by reaction of 2-(diethylamino)ethyl *p*-aminobenzoate with the appropriate substituted benzoyl chloride (method B). The new substituted benzoylaminobenzoic acids were prepared by acylation of aminobenzoic acid with the appropriate substituted benzoyl chloride. The characteristics of the new compounds are reported in Tables I-IV.

Pharmacology. The data related to smooth muscle relaxant activities are reported in Table V. It will be noted that, among the compounds tested, **39**, **42**, and **43** were shown to possess similar anticholinergic activity but considerably greater barium-induced contraction antagonizing properties as compared to scopolamine *N*-butyl bromide. Structural modifications of one of the most effective compounds, namely **42**, led to the synthesis of several derivatives, **40**, **41**, **44-48**, and **59**, whose effectiveness was inferior to that of the parent compound.

Quaternization of the same base with either MeI or MeBr resulted in compounds of comparable biological potency. Quaternization is accompanied by (a) increased toxicity; (b) increased anticholinergic properties; and (c) as a rule, decreased or increased effectiveness on histamine- and $BaCl_2$-induced spasm proportional to side-chain length in the R position. As far as some effects of quaternization are concerned (a and b), our results are in agreement with data in the literature.[2,3] Unlike toxicity, smooth muscle relaxant properties seem to be dependent upon side-chain length in the R position.

Table I



No.	R	% yield	Mp, °C	Formula[a]
1[b]	2-OCH_3	91	243-244	$C_{15}H_{13}NO_4$
2[b]	2-OC_2H_5	92	237-238	$C_{16}H_{15}NO_4$
3[b]	2-*n*-OC_4H_9	88	196-198	$C_{18}H_{19}NO_4$
4	2-*n*-OC_5H_{11}	89	187-189	$C_{19}H_{21}NO_4$
5	2-*n*-OC_8H_{17}	92	172-174	$C_{22}H_{27}NO_4$
6	3-*n*-OC_8H_{17}	85	220-221	$C_{22}H_{27}NO_4$
7	4-*n*-OC_8H_{17}	84	265-267	$C_{22}H_{27}NO_4$
8	2-$OCH_2C_6H_5$	88	211-212	$C_{21}H_{17}NO_4$
9	2-$O(CH_2)_2C_6H_5$	88	209-211	$C_{22}H_{19}NO_4$
10	2-$O(CH_2)_3C_6H_5$	90	196-198	$C_{23}H_{21}NO_4$

[a] All compounds were analyzed for C, H, and N. [b] Known; M. Ghelardoni and F. Russo, Belgian Patent 670,751 (1966); *Chem. Abstr.*, **65**, 16909*d* (1966).

Experimental Section

Chemical Procedures. Melting points were determined in an open capillary tube in a bath and are uncorrected. Ir spectra of the compounds in Nujol mulls were determined by means of a Perkin-Elmer 337 grating spectrometer. All spectra were consistent with the assigned structures.

Preparation of Substituted Benzoylaminobenzoic Acids (1-10; See Table I). These were made by refluxing the corresponding acid chloride with *p*-aminobenzoic acid in dioxane and pyridine for 2 hr. The precipitate was collected and extracted with 5% $NaHCO_3$. The solution was then acidified and the precipitate was filtered off and crystallized from EtOH.

Substituted 2-Aminoethyl *p*-Aminobenzoate (11-23, 25-30; See Table II). Method A (11, 12, 15, 16, 19-24, 28-30). The appropriate substituted *p*-benzamidobenzoic acid in $SOCl_2$ was refluxed gently for 2 hr. The excess $SOCl_2$ was removed under reduced pressure. The obtained acid chloride was added slowly with stirring to a solution of N-disubstituted 2-aminoethanol in pyridine. The mixture was stirred for 3 hr at 100°, cooled, and poured into H_2O. The solid was washed with H_2O and then crystallized.

Method B (13, 14, 17, 18, 25-27). A solution of 2-(diethylamino)ethyl *p*-aminobenzoate (0.1 mol) in H_2O (100 ml) was added slowly with stirring to a solution of 0.1 mol of the appropriate substituted benzoyl chloride. The mixture was maintained alkaline with 10% NaOH. The precipitate was collected and crystallized. When an oil was obtained, it was changed to the solid hydrochloride.

1-[*N*-(2-Octyloxybenzoyl)-*p*-aminobenzoyl]-4-diethylethylenediamine (24; See Table II). Method A was followed except that 2-diethylaminoethylamine was used, instead of N-disubstituted 2-aminoethanol.

Quaternary Salts 31-55 (Table III) and 57-59 (Table IV). These were prepared by dissolution of the corresponding bases in Et_2O followed by treating with MeI (or MeBr) at room temperature. The precipitate was removed by filtration and recrystallized.

2-(Diethylamino)ethyl (*o*-Octyloxy)benzoate (58; Table IV). Method A was followed except that (*o*-octyloxy)benzoyl chloride