lized from boiling acetone to give 0.31 g (20%) of **2**, mp 189.5–191° *Anal.* ($C_{13}H_{21}NO_3Cl_2$) C, H, N.

α-Chloromethylphenethylamine Hydrochloride (9). To 0.350 g (0.00264 mol) of benzylaziridine,[3] cooled to −10°, was added 1.5 ml of cold 18% aqueous HCl. The solution was evaporated to dryness at 60° (10 mm) to give 0.415 g (76.5%) of **9**, mp 175–180°. *Anal.* Calcd for $C_9H_{13}NCl_2$: Cl (ionic), 17.21; Cl (total), 34.42. Found: Cl (ionic), 16.98; Cl (total), 35.14.

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N-Substituted Derivatives of Erythromycylamine

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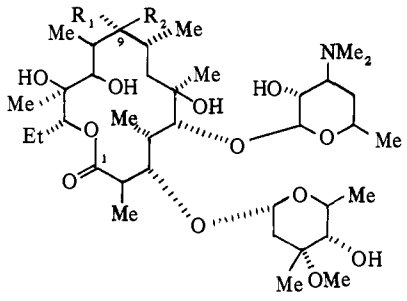
The antibacterial activities of derivatives of erythromycin A (**1**, $R_1R_2 = O$) are dependent on the nature of the substituent at C_9. Thus erythromycin itself,[1] the oxime,[2] and the hydrazone[3] are highly active against gram-positive bacteria, whereas the alcohol,[3] obtained by borohydride reduction of erythromycin, has much lower activity.

(9*S*)-Erythromycylamine[4] (**1**, $R_1 = H$; $R_2 = NH_2$) has antibacterial activity comparable to that of erythromycin



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itself, the 9*R* epimer being somewhat less active.[5] The present availability of (9*S*)-erythromycylamine in substantial quantity[6,7] has enabled us to explore the variation of antibacterial activity with N-substitution.

Simple *N*-alkyl derivatives of erythromycylamine are not easily prepared. For example, erythromycylamine reacts with aliphatic aldehydes with elimination of water to produce cyclic carbinolamine ethers[8] which are not readily reduced. Aromatic aldehydes, however, give the expected arylidene derivatives.[9,†] These Schiff bases readily revert to the aldehyde and the parent amine so that their activities cannot be determined by the usual methods. The arylidene derivatives were reduced by borohydride to a series of *N*-benzyl compounds. The mass spectra of these compounds showed good molecular ions and the presence of a benzyl group was confirmed in each case by the nmr spectrum.

Reaction of erythromycylamine with aliphatic ketones followed the pattern of the aromatic aldehydes giving imines (strong ir band at 1650 cm^{-1}) which were again readily reduced to secondary amines.

A group of *N*-alkyl derivatives bearing additional functional groups was obtained by addition of erythromycylamine to various α,β-unsaturated esters, nitriles, and ketones. In each case the nmr spectrum of the product indicated that the monoadduct only had been isolated and this was supported by elemental analysis. The adducts of erythromycylamine with ketones were reduced with borohydride to the corresponding hydroxyalkylamines which again gave good mass spectra.

The remaining compounds were prepared by reaction of erythromycylamine with various acid chlorides or anhydrides, isocyanates, and isothiocyanates as described in the Experimental Section. In each case, the nmr spectrum confirmed that reaction with only 1 equiv of the electrophile had occurred.

Antibacterial Activity. Compounds were tested *in vitro* against a range of bacteria by the agar gradient plate technique based on the method of Szybalski,[10] and the results are shown in Table I. The most striking effect on N-substitution is the marked reduction of antibacterial activity in those compounds (amides, sulfonamides ureas, and thioureas) which do not have a basic nitrogen at C_9. In most cases these compounds are virtually inactive. This is somewhat surprising in view of the high activity of erythromycin itself.

None of the new derivatives shows useful activity against either gram-negative organisms or the resistant strain of *Staphylococcus aureus* and in no case is the activity against sensitive organisms significantly greater than that of the parent compound.

Experimental Section‡

Erythromycylamine was prepared from erythromycin hydrazone[3] by nitrosation followed by borohydride reduction.[7]

***N*-(2,4,6-Trimethylbenzyl)erythromycylamine.** To a solution of *N*-(2,4,6-trimethylbenzylidene)erythromycylamine (7.75 g, 8.95 mmol) in MeOH (75 ml) at 0° was added solid $NaBH_4$ (1 g, excess). After standing 16 hr, the bulk of the MeOH was removed under reduced pressure and water added. The solution was extracted three times with CH_2Cl_2 and the dried ($MgSO_4$) extracts were evaporated to give an oil which crystallized from dry Et_2O giving 6.2 g. Dissolution in CH_2Cl_2, evaporation, and recrystallization from dry Et_2O gave 5.07 g (65%), mp 158–160°, pure by tlc. The ir spectrum showed no C=N. *Anal.* ($C_{47}H_{82}N_2O_{12}$) C, H, N.

N-Benzyl- and *N*-(*p*-nitrobenzyl)erythromycylamine were prepared similarly.

***N*-Isopropylerythromycylamine.** A solution of erythromycylamine (5.0 g) in Me_2CO (40 ml) was heated under reflux for 60 hr. Tlc showed almost complete conversion to a faster running compound. The excess Me_2CO was evaporated and to a solution of the resulting oil in MeOH (40 ml) at 0° was added $NaBH_4$ (600 mg, excess) and the solution stirred for 2 hr. The bulk of the solvent was evaporated, water added, and the solution extracted three times

†Details for the preparation of benzylideneerythromycylamine were kindly supplied by Dr. E. H. Massey, Eli Lilly & Co., Indianapolis, Ind.

‡Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Ir spectra were determined with a Perkin-Elmer 457 grating infrared spectrophotometer and nmr spectra with a Varian A-60A spectrometer. Ir and nmr spectra of all new compounds supported the proposed structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values. All tlc was carried out on silica gel F_{254} (Merck) using 3:1 MeOH–DMF as solvent system. In this system, erythromycylamine runs near the bottom of the plate and N-substituted derivatives have R_f's of about 0.6.

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

			Minimum inhibitory concentration, μg/ml				
			Staph. aureus			*E. coli*	*Proteus morganii*
R_2	Mp, °C	Analyses	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]