Antibacterial Activity of 9(S)-Erythromycylamine-Aldehyde Condensation Products

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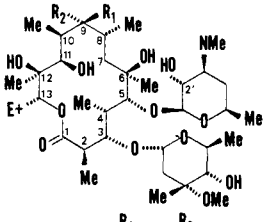
In vitro and *in vivo* antibacterial activities of 9(*S*)-erythromycylamine (**3**), a series of aromatic and aliphatic aldehyde condensation products (**5**, **7a**,**b**) of **3**, and 9(*R*)-erythromycylamine (**4**) are reported. Therapy of experimental *Streptococcus pyogenes* infections in mice coupled with pharmacokinetic studies in dogs showed that several arylidene derivatives, particularly the *N*-benzylidene **8**, were absorbed better than erythromycin (**1**) or **3** after oral administration. Although **8** compared favorably with erythromycin in all *in vivo* laboratory tests, it gave lower blood concentrations (po) in human subjects.

The importance of the C_9 carbonyl function for antibacterial activity in erythromycin (**1**)[1,2] is demonstrated by the very low activity of 9(*S*)-dihydroerythromycin (**2**).[3] Acid-catalyzed dehydration of **1** to 8,9-anhydroerythromycin 6,9-hemiketal[4] or to anhydroerythromycin[1] (anhydroerythromycin 6,9,12-spiroketal) likewise results in virtually a complete loss of activity. However, the recent observation that 9(*S*)-erythromycylamine (**3**),‡[,5,6] the C_9-amine counterpart of **2**, has 45% the *in vitro* antibacterial activity of **1** against *Staphylococcus aureus* isolates (see Table II) suggests that the carbonyl or other sp^2 hybridized function at C_9 is not as necessary for activity as had been thought previously.[2]

To find a compound with activity similar to **3**, but that is absorbed better when administered orally, we have prepared a number of aldehyde condensation products[7,8] of **3** and have evaluated them in mice and dogs. Unfortunately, our animal models have failed to correctly predict the outcome of studies in humans. Also, we have prepared and determined the antibacterial activity of 9(*R*)-erythromycylamine (**4**).[9]

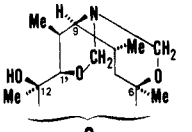
Chemistry. In an earlier communication,[5] we described the physical properties of **3** obtained from the catalytic reduction of erythromycin 9-oxime in water or glacial acetic acid using PtO_2. Epimer **4** was isolated as a minor (<10%) product. Subsequent to our work, an improved chemical synthesis of **3**, with no trace of **4**, from erythromycin 9-hydrazone has been reported by Wildsmith.[10,§] High-pressure hydrogenation of the 9-oxime using excess Raney nickel gave **4** as the predominant product.

Arylaldehydes react with **3** to form the expected *N*-arylidene derivatives. Their uv absorption in the 250–320-nm region and ir bands around 1640 cm^{-1} are consistent with the conjugated azomethine structure **5**.



	R_1	R_2
1	R_1,R_2=O	
2	-OH	-H
3	$-NH_2$	-H
4	-H	$-NH_2$
5	-N=CHAr	-H

Alkyl- and aralkylaldehydes do not condense with the C_9-amino group in **3** to form alkylidenes but yield cyclic carbinolamine ethers.[12] These products arise from the intramolecular cyclization of the intermediate alkylidene or carbinolamine and the hydroxyl at either C_6, C_{11}, or C_{12}. In the case of formaldehyde, 2 mol of aldehyde is added to form a bisadduct **6**. Acetaldehyde and higher homologs form monoadducts which show no ir absorption in the 1620–1650-cm^{-1} region (C=N) and are not reduced to secondary amines by sodium borohydride. Although the structures are not known with certainty, the monoadducts are thought to be either tetrahydro-1,3-oxazines **7a** involving the C_{11} oxygen or hexahydro-1,3-oxazapines **7b** involving the C_6 oxygen. Oxazapine structures including C_{12} were ruled out because the crude 9-amino compound‡ obtained from the 9-oxime of erythromycin B,[2] which is the same as **1** except that the hydroxyl at C_{12} is replaced by a hydrogen, also reacts with excess formaldehyde to give a bisadduct.



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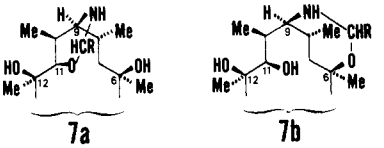

7a **7b**

R=alkyl or aralkyl

Microbiological Results and Discussion. The compounds prepared for this study were compared initially in the therapy of experimental *Streptococcus pyogenes* C203 infections in mice. The MIC values for this organism, the ED_{50} values for oral and subcutaneous administration, and pertinent physical-chemical data for each compound tested are summarized in Table I. These data indicated that 9(*S*)-erythromycylamine (**3**) was several times more active than its 9(*R*) epimer **4**, and no further modification or testing of **4** was done. With MIC values in the 0.15–1.25-μg/ml range, the aryl and aliphatic aldehyde derivatives of **3** vary from as active as to somewhat less active than the parent amine (0.15 μg/ml). However, the ED_{50} values for oral administration of the arylidene derivatives **8**–**15** are lower in general than the ED_{50}'s for the alkyl and aralkyl adducts, suggesting that they are more completely absorbed. After oral dosing to infected mice the *N*-benzylidene **8** and the *N*-salicylidene **9** derivatives showed better *in vivo* activity than other adducts of both classes, the amine **3**, erythromycin, and 2′-propionylerythromycin.[13,**]

† Personally acquainted with him since Volume I of his—now our—Journal, one of us, K. G., takes pleasure in paying tribute to Alfred Burger as a scientist and as a person.

‡ Previously,[5] this compound was called erythromycylamine. The C_9 configuration was assigned on the basis of unpublished nmr data on the descladinose and erythronolide species by P. V. Demarco, Lilly Research Laboratories.

§ This group[11] also has compared the antimicrobial activity of a series of N-substituted derivatives of **3**, which were obtained by $NaBH_4$ reduction of Schiff bases similar to those reported here.

‡ Since this compound was prepared in the same way as **3**, it was assumed to have the 9(*S*) configuration.

** Marketed as the lauryl sulfate salt under the tradename Ilosone (Eli Lilly and Co.).

Table I. Aldehyde Condensation Products of 9(*S*)-Erythromycylamine

No.	Derivative	Formula[a]	Mp, °C	M+,[b] *m/e*	Mouse therapy[c] ED_{50} po, mg/kg × 2[c]	Mouse therapy[c] ED_{50} sc, mg/kg × 2	MIC,[l] μg/ml
		Arylidenes					
8	*N*-Benzylidene	$C_{44}H_{74}N_2O_{12}\cdot H_2O$	142–146[d]	822	15.6	3.6	0.4
9	*N*-Salicylidene	$C_{44}H_{74}N_2O_{13}\cdot H_2O$	153–155[d]	838	10.4	2.4	0.6
10	*N*-1-Naphthylmethylene	$C_{48}H_{76}N_2O_{12}\cdot H_2O$	140–146[e]	872	28.6	2.6	0.6
11	*N*-4-Methylbenzylidene	$C_{45}H_{76}N_2O_{12}$	141–143[d]	836	18.2	0.8	0.15
12	*N*-4-Carboxybenzylidene	$C_{45}H_{74}N_2O_{14}$	212–215 dec[f]	866	67.5	1.5	1.25
13	*N*-4-Methoxybenzylidene	$C_{45}H_{76}N_2O_{13}$	Amorphous	852	20.8	1.0	0.15
14	*N*-4-Chlorobenzylidene	$C_{44}H_{73}ClN_2O_{12}$[g]	Amorphous	856	19.5	0.9	0.15
15	*N*-3-Pyridylmethylene	$C_{43}H_{73}N_3O_{12}$	Amorphous	823	36.4	1.0	0.15
		Aliphatic Adducts[h]					
6	Formaldehyde[i]	$C_{39}H_{70}N_2O_{12}$	215	758	31.0	2.6	0.6
16	Acetaldehyde	$C_{39}H_{72}N_2O_{12}$	196–197	760	31.2	2.6	0.3
17	Propionaldehyde	$C_{40}H_{74}N_2O_{12}$	195–197	774	36.4	1.6	0.3
18	Butyraldehyde	$C_{41}H_{76}N_2O_{12}$	184–185	788	23.4	1.3	0.3
19	Valeraldehyde	$C_{42}H_{78}N_2O_{12}$	175–177	802	26.0	1.6	0.6
20	Isovaleraldehyde	$C_{42}H_{78}N_2O_{12}$	194–195	802	20.8	2.6	1.25
21	Phenylacetaldehyde	$C_{45}H_{76}N_2O_{12}$	211–213	836	18.2	1.3	1.25
22	Phenylpropargylaldehyde	$C_{46}H_{74}N_2O_{12}$	211–212	846	26.0	1.6	0.6
23	Pivaldehyde	$C_{42}H_{78}N_2O_{12}$	100	802	9.8	1.0	0.15
		For Reference					
1	Erythromycin[j]				46.7	4.9	0.05
3	9(*S*)-Erythromycylamine	*c*			26.0	2.6	0.15
4	9(*R*)-Erythromycylamine	*c*			>42.0	6.0	2.5
	2′-Propionylerythromycin[k]				23.4	3.9	

[a] All compounds were analyzed for C, H, and N and were within ±0.3% of calculated values and had ir, uv, and nmr spectra compatible with their assigned structure. [b] Molecular weight by mass spectrometry. [c] See Experimental Section. [d] Recrystallized from *i*-PrOH–H_2O. [e] Recrystallized from Et_2O–MeOH. [f] Recrystallized from *i*-PrOH. [g] *Anal.* C, H, N, Cl. [h] Adducts **16–23** recrystallized from Et_2O. [i] Bisadduct, recrystallized from EtOH. [j] As the glucoheptonate salt. [k] Met all published specifications.[12] [l] Against infecting organism, *S. pyogenes*.

Table II. Summary of *in Vitro* Activity against 29 *Staphylococcus aureus* Isolates

Compound[a]	Mean MIC,[b] μg/ml	Range, μg/ml Low	Range, μg/ml High
Erythromycin (**1**)	0.53	0.1	25
9(*S*)-Erythromycylamine (**3**)	1.26	0.2	25
N-Benzylidene-9(*S*)-erythromycylamine	0.98	0.2	12.5
N-Salicylidene-9(*S*)-erythromycylamine	0.79	0.2	12.5

[a] All compounds were dissolved in 20% DMF. [b] Geometric mean.

Because of their favorable activity in mouse therapy experiments, **8** and **9** were compared *in vitro* with **3** and erythromycin against 29 strains of *Staphylococcus aureus*. The MIC values obtained by the ICS agar dilution technique (see Experimental Section) are summarized in Table II. The antibacterial activities of **3**, **8**, and **9** are essentially the same, about 40–60% that of erythromycin.

Single 25 mg/kg doses of erythromycin (**1**), 9(*S*)-erythromycylamine (**3**), the *N*-benzylidene **8** and *N*-salicylidene **9** derivatives, and three aliphatic adducts (**20**, **21**, and **23**) were administered orally to six fasting dogs in a crossover blood level study. All gave measurable blood concentrations (see Table III). Both **3** and **8** gave levels similar to those given by erythromycin, and at 4–6 hr the blood concentrations of **3** and **8** were higher. The amounts of **8** and erythromycin excreted in the urine (0–6 hr) were nearly the same.

In further studies on the *N*-benzylidene in dogs using daily oral doses of 25, 50, and 200 mg/kg over a 14-day period sustained blood concentrations were observed. No toxic effects or changes in blood chemistry were noted in these animals.

From the *in vivo* and *in vitro* data the *N*-benzylidene derivative appeared to be a suitable candidate for study in humans. Although less active *in vitro* than erythromycin, it gave blood concentrations in dogs that were definitely greater than the MIC's for most susceptible organisms. Unfortunately, in a crossover study in five human volunteers in which four 250-mg oral doses of the *N*-benzylidene

Table III. Mean Blood Concentrations (μg/ml)[a] of Antibiotic Activity in Six Dogs[b] after an Oral Dose of 25 mg/kg

Compd	Hours after dose 0.5	1	1.5	2	3	4	6	% of dose in urine, 0–6 hr
1	2.4	2.7	1.2	0.9	1.2	0.4	0.2	4.58
3	0.4	1.0	1.4	0.7	0.6	0.6	0.6	1.90
8	0.5	2.3	1.6	2.0	1.0	1.2	0.7	4.89
9	0.1	0.1	0.4	0.4	0.6	0.5	0.4	1.74
20	0.2	0.6	0.8	0.5	0.7	0.3	0.3	1.48
21	0.9	1.4	1.0	1.0	0.5	0.6	0.4	2.54
23	<0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.81

[a] Assayed by disk plate method against *S. lutea* using each compound as its own standard. [b] Fasting state.

derivative 8 and erythromycin were given at 6-hr intervals, the *N*-benzylidene failed to give blood concentrations greater than 0.06 μg/ml after the fourth dose. Erythromycin gave peak levels of 0.3–1.8 μg/ml in the same subjects.††

Experimental Section

Microbiological Methods and Materials. Agar dilution MIC values were determined using Mueller-Hinton agar by the method recommended by the International Collaborative Study as described by Ericsson and Sherris.[14] Disk diffusion studies were performed by the Bauer-Kirby method.[15] Experimental infections in McAllister strain mice (11–13 g) induced by intraperitoneal administration of *Streptococcus pyogenes* C203 were treated 1 and 5 hr postinfection by subcutaneous or oral administration (stomach tube) of the test antibiotic. Groups of eight mice for each of five dose levels were observed for 7 days. ED_{50} values were calculated by the method of Reed and Muench.[16] Antibiotic concentrations in dog serum were assayed by disk plate using *Sarcina lutea* PC1-1001-FDA against standard curves of the test compound diluted in horse serum. Urine samples from dogs were assayed using a standard curve for the test compound diluted in 0.85% saline.

Chemical Methods and Materials. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Melting points of amorphous products are not reported. Ir spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer, uv spectra on a Cary Model 15, and mass spectra on a CEC-110 high-resolution spectrometer. Potentiometric titrations were done in 66% DMF–H_2O. Microanalyses are ±0.3% of theory. Tlc was performed on precoated EM Laboratories Silica Gel F-254 plates using (A) MeOH–DMF (3:1) or (B) EtOAc–Et_2NH–H_2O (50:1:1).

9(*S*)-Erythromycylamine (3). Method A. Erythromycin 9-ketoxime[5] (8.0 g, 11 mmol) dissolved in 360 ml of H_2O containing 4.2 g (23 mmol) of δ-gluconolactone was hydrogenated at room temperature (16 hr, 700 psi) using 2.5 g of PtO_2. After the catalyst had been removed, the resultant clear solution was adjusted with 1 *N* NaOH to pH 9.5 and extracted with CH_2Cl_2 (4 × 80 ml). The combined CH_2Cl_2 solutions were dried (Na_2SO_4) and evaporated to a white amorphous foam, which crystallized from 25 ml of ether to give 5.8 g of **3**: mp 132–137°; R_f 0.3–0.4 (system A), 0.5 (system B, run plate twice); *m/e* 734; pK_a 8.8 and 9.8; ir ($CHCl_3$) 1720 cm^{-1} (lactone carbonyl). *Anal.* ($C_{37}H_{70}N_2O_{12}$) C, H, N, O.

Method B. Erythromycin 9-ketoxime (17 g, 23 mmol) dissolved in 225 ml of glacial HOAc containing 12.5 g of prereduced PtO_2 was hydrogenated (700 psi, room temperature) for 48 hr. Removal of the catalyst and lyophilization of the clear HOAc solution gave amorphous **3** as the acetate salt. A CH_2Cl_2 solution of the salt was washed with saturated $NaHCO_3$, and crystalline **3** (15 g) was isolated as described above.

9(*R*)-Erythromycylamine (4). Erythromycin 9-ketoxime (2.5 g, 3 mmol) dissolved in 450 ml of MeOH was hydrogenated (16 hr, 1000 psi, 1000 rpm of stirring) using 50 g of Raney nickel. Removal of the catalyst and MeOH gave an amorphous white solid, which crystallized from Et_2O to give 1.8 g of **4**: mp 178–181°; R_f 0.2 (system A), 0.2 (system B, run plate twice); *m/e* 734; pK_a 8.4 and 9.9. *Anal.* ($C_{37}H_{70}N_2O_{12}$) C, H, N, O.

9(*S*)-Erythromycylamine-Bisformaldehyde Adduct 6. A solution of 1.0 g (1.4 mmol) of **3** and 10 ml of 37% aqueous H_2CO in 25 ml of EtOH was maintained at room temperature for 6 hr. The oily residue obtained upon evaporation of the EtOH was dissolved in Et_2O. The Et_2O solution was dried (Na_2SO_4) and evaporated to give an amorphous residue, which crystallized from 3–5 ml of anhydrous EtOH to give 0.8 g of **6** as needles: mp 213–215°. See Table I for other physical data.

Aliphatic and Aralkyl Aldehyde Adducts 16–23. Solutions of **3** (1.0 g, 1.4 mmol) and the appropriate aldehyde (1.5 mmol) in 25–30 ml of EtOH were allowed to stand for 12–24 hr. Tlc (system A) indicated reactions were nearly complete after 30 min; R_f's of adducts are 1.5–2 times the R_f of **3**. The white amorphous foams obtained on removal of the EtOH were crystallized from Et_2O to give the adducts in 80–90% yields. See Table I for physical constants.

Arylidene Derivatives 8–15. The arylaldehyde (5.0 mmol) was added to a suspension of 3.5 g (4.7 mmol) of **3** in hot *i*-PrOH. The mixture usually became homogeneous after 20–30 min and heating (60–70°) was continued for 2–3 hr. Water was added slowly until precipitation was complete. Yields of product after drying at 50° (vacuum) were 80–95%. Tlc (system A) shows the products to be homogeneous. In some cases the products were recrystallized from MeOH–H_2O, *i*-PrOH, or *i*-PrOH–H_2O (see Table I).

Bisformaldehyde Adduct of Erythromycylamine B. The ketoxime of erythromycin B (1.0 g, 1.4 mmol) was prepared by the same method as for erythromycin 9-ketoxime.[5] Without purification, the oxime (~1 g) was catalytically hydrogenated in 100 ml of glacial HOAc using 1 g of PtO_2. As described above for **3**, the amine B was isolated as an amorphous white foam: *m/e* 718; pK_a 8.3 and 9.7. *Anal.* ($C_{37}H_{70}N_2O_{11}$) C, H, N.

A solution of 21 mg of amine B and 0.25 ml of 38% aqueous formaldehyde in 5 ml of EtOH was allowed to stand for 16 hr at room temperature. The EtOH was removed at reduced pressure. The wet residue was repeatedly dissolved in benzene and evaporated to dryness to give 20 mg of the diformaldehyde adduct as an amorphous white solid: *m/e* 742. *Anal.* ($C_{39}H_{70}N_2O_{11}$) C, H, N.

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†† Both compounds were given as enteric-coated minitablets. Unpublished human blood-level data were supplied by Dr. C. F. Speirs, Lilly Research Centre, Erl Wood Manor, Surrey, England.