Motilides, Macrolides with Gastrointestinal Motor Stimulating Activity. II. Quaternary *N*-Substituted Derivatives of 8,9-Anhydroerythromycin A 6,9-Hemiacetal and 9,9-Dihydroerythromycin A 6,9-Epoxide

Toshiaki SUNAZUKA,[a] Kazuo TSUZUKI,[a] Shogo MARUI,[a] Hajime TOYODA,[a] Satoshi ŌMURA,*[,a] Nobuhiro INATOMI,[b] and Zen ITOH[b]

The Kitasato Institute and School of Pharmaceutical Science of Kitasato University,[a] Minato-ku, Tokyo 108, Japan and College of Medical Technology of Gunma University,[b] Maebashi, Gunma 371, Japan. *Received February 10, 1989*

A series of quaternary ammonium derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal (1) and 9,9-dihydroerythromycin A 6,9-epoxide (2) has been prepared and tested for antimicrobial activity and gastrointestinal motor stimulating (GMS) activity in the dog (*in vivo*). The GMS activity is enhanced markedly when small alkyl halides and unsaturated alkyl halides such as allyl bromide and propargyl bromide are added to the dimethylamino group of 1. Among them, *N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal bromide (3) exhibits GMS activity 2890 times stronger than that of erythromycin A and is completely devoid of antimicrobial activity. The potency of 3 is comparable to that of synthetic motilin both *in vitro* and *in vivo*.

Keywords motilide; macrolide; motilin; gastrointestinal motor stimulating activity; interdigestive migrating contraction; 8,9-anhydroerythromycin A 6,9-hemiacetal; *N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal bromide; *N*-propargyl-9,9-dihydroerythromycin A 6,9-epoxide bromide

Introduction

Subsequent to the findings that erythromycin A (EM-A) has an activity to induce interdigestive migrating contractions in the gastrointestinal tract in the dog,[1] a series of chemical modifications of EM-A was initiated in these laboratories to search for derivatives having stronger gastrointestinal motor stimulating (GMS) activity and no antimicrobial activity. Our initial investigation[2] afforded 8,9-anhydroerythromycin A 6,9-hemiacetal (**1**) and de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (**2**), whose GMS activities were 10 times and 248 times more potent, respectively, than that of EM-A. Moreover, they were virtually devoid of antimicrobial activity. These findings strongly suggested that the dimethylamino group of **1** plays an important role as an active site responsible for GMS activity. In this report we describe further modification of the dimethylamino group of **1** which led to a series of quaternary ammonium derivatives with greatly increased potencies. Among them, *N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal bromide (**3**), was the most potent derivative, exhibiting potency comparable to that of synthetic motilin both *in vivo* and *in vitro*.

Chemistry

The compounds prepared for this study are listed in Tables I, II and III. Their syntheses are shown in Charts 1 and 2. Alkylation of the dimethylamino group on the desosamine moiety of **1** was achieved with various alkyl halides by three methods, according to their reactivities. Alkylation of **1** with an excess of highly reactive alkyl halide in $CHCl_3$ at ambient temperature or at 60 °C (method A) resulted in quantitative conversion to the quaternary ammonium derivative. An alternative alkylation procedure (method B) is exemplified by the preparation of the 2-hydroxyethyl quaternary ammonium derivative (**16**) on treatment of a $CHCl_3$ solution of **1** with excess 2-bromoethanol in the presence of *N*, *N*-diisopropylethylamine at reflux. This procedure was also used for the preparation of the methoxycarbonylmethyl quaternary ammonium derivative (**18**), since the attempted synthesis by method A provided anhydroerythromycin A instead of the desired ammonium derivative. Alkylation of **1** with relatively unreactive alkyl halides such as 2-iodopropane could not be achieved by methods A and B. Alkylation of de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal[2] with an excess of 2-iodopropane in $CHCl_3$ in the presence of *N*,*N*-diisopropylethyl-amine at reflux, followed by methylation with MeI, provided the isopropyl quaternary ammonium derivative (**20**) (method C). On the other hand, syntheses of quaternary ammonium derivatives bearing different alkyl groups were achieved from de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal[2] and bis-de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal[2] by successive alkylation with the corresponding alkyl halides in a similar manner to that described above. The effect of the accompanying ion (counter ion) of quaternary ammonium derivatives upon GMS activity was also investigated. In the case of **5**, which has GMS activity 110 times stronger than that of EM-A, treatment with Ag_2O in 50% aqueous MeOH led to translactonization[3] resulting in a 12-membered lactone (*cf.* **85**, Chart 3 in the accompanying paper) accompanied with the conversion of the counter ion ($I^- \rightarrow OH^-$). On treatment with Ag_2O in AcOH–MeOH–H_2O, the counter ion of **5** was converted from I^- to OAc^- to obtain **35**.

The structures of the quaternary ammonium derivatives were confirmed by carbon-13 nuclear magnetic resonance (^{13}C-NMR) (Table VI) and high-resolution fast atom bombardment mass spectroscopy (HR-FAB-MS).

Biological Results and Discussion

In the previous investigation, it was found that the dimethylamino group on the desosamine moiety of **1** has distinctive roles in the structure activity relationships involving GMS activity, and modification of the amine was useful for enhancement of GMS activity as well as for the separation of GMS activity from antimicrobial activity. Subsequently, a second-stage chemical modification of the dimethylamino group was conducted to afford a series of quaternary ammonium derivatives by alkylation of **1** with various alkyl halides. The results are reported here.

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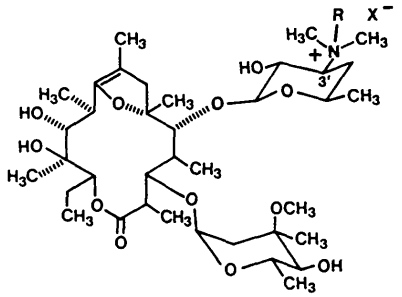
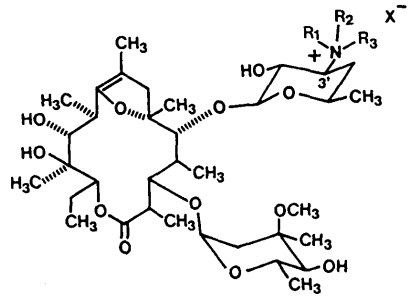
TABLE I. Physical Properties of Quaternary Ammonium Derivatives of **1**



No.	R	X	Method[a]	Yield (%)[b]	$[\alpha]_D$[c] (c=1.0, CH_3OH)	HR-FAB-MS[d] Calcd	HR-FAB-MS[d] Found
3	$CH_2C\equiv CH$	Br	A	48	−31.0° (22)	754.473	754.473
4	CH_3	I	A	54	−28.6° (23)	730.473	730.473
5	C_2H_5	I	A	60	−25.4° (23)	744.489	744.491
34	C_2H_5	Br	A	82	−31.2° (23)	744.489	744.490
35	C_2H_5	OAc			−28.5° (23)		
6	n-C_3H_7	I	A	48	−28.4° (23)	758.505	758.505
7	n-C_4H_9	I	A	64	−29.4° (24)	772.520	772.520
8	$CH_2C_6H_5$	Cl	A	52	−39.6° (23)	806.505	806.504
9	CH_2CH_2F	Br	A	76	−26.0° (22)	762.480	762.480
10	$CH_2C\equiv N$	Br	A	94	−40.4° (22)	755.469	755.470
11	CH_2–◁	Br	A	81	−24.4° (24)	770.505	770.505
12	$CH_2CH_2CH=CH_2$	Br	A	56	−32.2° (25)	770.505	770.504
13	$CH_2CH=CHCH_3$	Br	A	98	−29.6° (24)	770.505	770.504
14	$CH_2C(Br)=CH_2$	Br	A	50	−26.2° (24)	834.399	834.397
15	$CH_2C\equiv CCH_3$	Br	A	100	−17.6° (25)	768.489	768.491
16	CH_2CH_2OH	Br	B	84	−26.4° (23)	760.484	760.492
17	$CH_2CH=CH_2$	Br	B	76	−25.8° (23)	756.489	756.489
18	$CH_2CO_2CH_3$	Br	B	80	−30.0° (22)	788.479	788.489
19	CH_2CO_2H	Br	B	71	−31.8° (22)	774.463	774.463
20	$CH(CH_3)_2$	I	C	23	−26.4° (24)	758.505	758.507
21	$CH_2CH(CH_3)_2$	I	C	12	−26.0° (24)	772.520	772.521
22	$CH_2CH_2C\equiv CH$	Br	C	14	−19.2° (24)	768.489	768.491

a) See Experimental for details of methods A—C. *b*) Overall yield from **1**. *c*) ( ); operating temperature (°C). *d*) $[M^+ - X]$ (X = counter ion) was observed in FAM-MS.

TABLE II. Physical Properties of Quaternary Ammonium Derivatives of **1**



No.	R_1	R_2	R_3	X	$[\alpha]_D$[a] (c=1.0, CH_3OH)	HR-FAB-MS[b] Calcd	HR-FAB-MS[b] Found
23	CH_3	C_2H_5	C_2H_5	I	−27.0° (23)	758.505	758.505
24	C_2H_5	C_2H_5	C_2H_5	I	−24.2° (22)	772.520	772.520
25	$-(CH_2)_4-$		CH_3	I	−27.0° (23)	756.489	756.488
26	$-(CH_2)_4-$		C_2H_5	I	−27.0° (22)	770.505	770.505
27	$-(CH_2)_5-$		CH_3	I	−27.0° (22)	770.505	770.506
28	$-(CH_2)_5-$		C_2H_5	I	−26.6° (22)	784.520	784.521
29	CH_3	$CH_2CH=CH_2$	$CH_2CH=CH_2$	Br	−24.4° (24)	782.505	782.504
30	CH_3	$CH_2C\equiv CH$	$CH_2C\equiv CH$	Br	−20.5 (24)	778.473	778.473
31	$CH_2CH=CH_2$	$CH_2CH=CH_2$	$CH_2CH=CH_2$	Br	−13.3° (24)	808.520	808.523
32	$CH_2CH=CH_2$	$CH_2CH=CH_2$	$CH_2C\equiv CH$	Br	−18.0° (24)	806.505	806.505
33	$CH_2C\equiv CH$	$CH_2C\equiv CH$	$CH_2C\equiv CH$	Br	−18.4° (24)	802.473	802.472

a) ( ), operating temperature (°C). *b*) $[M^+ - X]$ (X: counter ion) was observed in FAB-MS.

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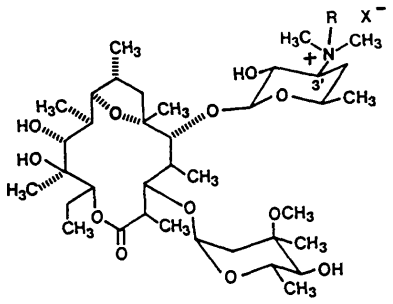
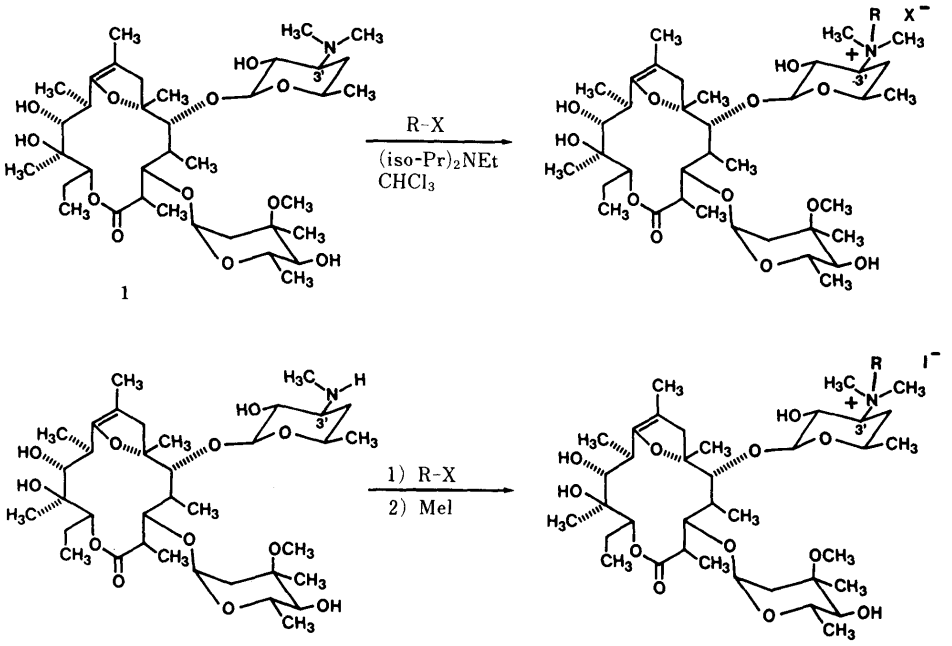
TABLE III. Physical Properties of Quaternary Ammonium Derivatives of **2**



No.	R	X	Method[a]	Yield (%)[b]	$[\alpha]_D$[c] (c=1.0, CH_3OH)	HR-FAB-MS[d]	
						Calcd	Found
36	CH_3	I	A	71	−38.0° (23)	732.489	732.488
37	C_2H_5	I	A	74	−35.2° (23)	746.505	746.503
38	n-C_3H_7	I	A	70	−40.6° (23)	760.520	760.522
39	n-C_4H_9	I	A	76	−40.6° (23)	774.536	744.536
40	$CH_2CH=CH_2$	Br	A	90	−38.4° (22)	758.505	758.504
41	$CH_2C\equiv CH$	Br	A	84	−41.2° (22)	756.489	756.489
42	$CH_2C_6H_5$	Cl	A	81	−47.4° (24)	808.520	808.518
43	CH_2CH_2OH	Br	A	67	−41.8° (25)	762.499	762.499
44	CH_2CH_2F	Br	A	37	−38.4° (24)	764.495	764.495
45	$CH_2C\equiv N$	Br	A	64	−42.6° (25)	757.484	757.489
46	CH_2-◁	Br	A	86	−37.0° (24)	772.520	772.519
47	$CH_2CH_2CH=CH_2$	Br	A	63	−37.6° (24)	772.520	772.520

a) See Experimental for details of method A. *b*) Overall yield from **2**. *c*) Samples were dissolved in MeOH, ( ): operating temperature (°C). *d*) $[M^+-X]$ (X: counter ion) was observed in FAB-MS.



de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal

Chart 1

The quaternary ammonium derivatives in Tables IV and V were evaluated for their *in vitro* antimicrobial activity (minimum inhibitory concentration (MIC)) and *in vivo* GMS activity in the dog. Their antimicrobial activities had been almost completely lost, while the GMS activities were greatly increased. Among the derivatives bearing saturated alkyl halides, addition of methyl iodide (**4**)[4] successfully increased GMS activity to 21 times that of EM-A, namely, about 2 times that of **1**, while addition of ethyl iodide (**5**) increased the activity to 111 times that of EM-A in a single step. Elongation of the *N*-alkyl group from ethyl to propyl (**6**) and butyl (**7**) groups rather decreased the GMS activity. Addition of an isopropyl group (**20**) increased the activity to 51 times that of EM-A, while the isobutyl derivative (**21**) exhibited GMS activity comparable to that of **1**. Instead of ethyl iodide, addition of substituted alkyl halides such as 1-

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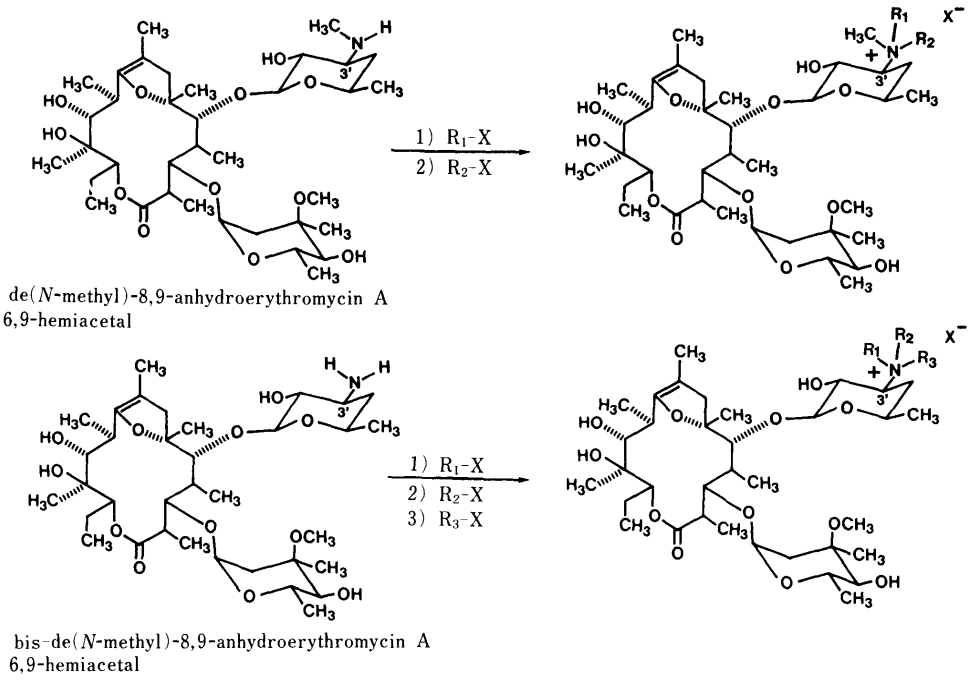

de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal

bis-de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal

Chart 2

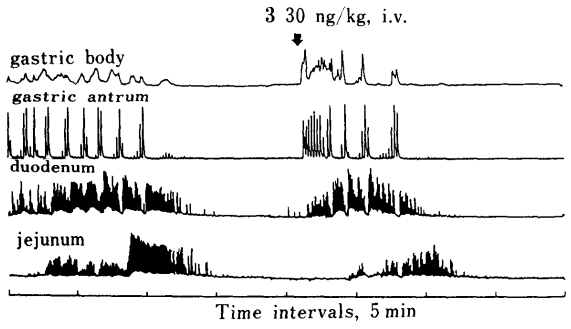

Fig. 1. Effect of an Intravenous Bolus Injection of **3** (30 ng/kg) at 10 min after the Termination of the Natural Interdigestive Contractions in a Conscious Dog

Compound **3** induced a series of strong contractions in the stomach, which migrated in a caudal direction along the small intestine. This contractile pattern is quite similar to the natural interdigestive contractions, which are seen on the left-hand side of this figure.

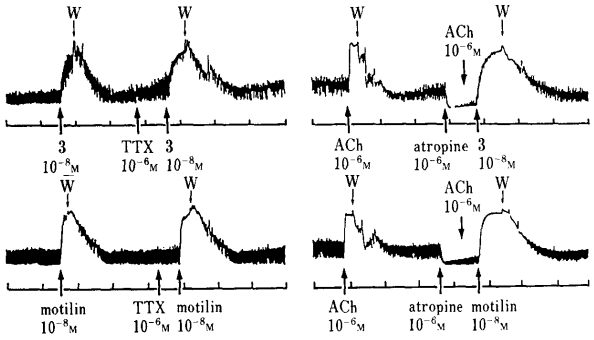

Fig. 2. Comparison of Contractile Responses of the Isolated Rabbit Duodenum in a Longitudial Axis to **3**, Synthetic Motilin, and Acetylcholine (ACh) *in Vitro*

The contractile response induced by **3** (10^{-8} M) was phasic with a gradual tonal increase while that induced by ACh (10^{-6} M) was a rapid tonic contraction. The effect of **3** on smooth muscle contraction was not inhibited by the pretreatment of the muscle preparation with tetrodotoxin (TTX, 10^{-6} M) or atropine (Atr, 10^{-6} M). The minimum effective concentration of **3** measured in this system was found to be 10^{-9} M, which was comparable to that of synthetic motilin.[5] W with arrows indicates repeated washing of the preparation.

bromo-2-fluoroethane (**9**), 2-bromoethanol (**16**), methyl bromoacetate (**18**), and bromoacetic acid (**19**) decreased the activity. On the other hand, addition of unsaturated alkyl halides such as allyl bromide (**17**) and propargyl bromide (**3**) greatly increased the GMS activity to 256 times and 2890 times that of EM-A, respectively. However, their homologs such as **8**, **10**—**15**, and **22** exhibited decreased activities. Combinations of three alkyl groups in the quaternary ammonium group were then examined. The derivatives bearing two or three allyl groups and propargyl groups exhibited decreased activites when compared with **3** and **17**. The effect of counter ions on GMS activity was also examined. The activity of the bromide (**34**) was comparable to that of the iodide (**5**), whereas the activity of the acetate (**35**) was only one-half that of **5**. Finally, similar modification was applied to 9,9-dihydroerythromycin A 6,9-epoxide (**2**), which is stable to acid, and the GMS activities exhibited by allyl and propargyl derivatives were also increased to a great extent. However, the magnitude of the increase did not reach the maximum achieved by modifying **1**. When EM-A was similarly modified, the increase in GMS activity remained very small.

To summarize the results of the present study, GMS activity seems to be affected by the size and the shape of the electron cloud around the nitrogen atom of the quaternary ammonium group and is not correlated to the electrostatic effect of the substituted alkyl group. The fact that quaternary ammonium type derivatives of **1** and **2** exhibited much more potent activity than those of EM-A may indicate extreme importance of the stereochemical structure of the aglycone moiety.

When **3**, the most potent derivative, was given as an intravenous bolus dose of 30 ng/kg to conscious dogs, a

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Table IV. Antimicrobial Activity (MIC) and GMS Activity in the Dog (*in Vivo*) of Quaternary Ammonium Derivatives of **1**

Compound No.	Antimicrobial activity: MIC[a] (μg/ml)					GMS activity[b]
	SA	BS	BC	EC	KP	
EM-A	0.2	0.1	0.1	12.5	6.25	1
1	50	25	25	>100	>100	10
3	100	100	100	>100	>100	2890
4	>100	>100	>100	>100	>100	21
5	>100	>100	>100	>100	>100	111
34	>100	>100	>100	>100	>100	115
35	50	100	100	>100	>100	61
6	>100	>100	100	>100	>100	9
7	100	>100	>100	>100	>100	<1
8	100	50	100	>100	>100	<1
9	>100	>100	>100	>100	>100	70
10	>100	>100	>100	>100	>100	82
11	100	>100	>100	>100	>100	18
12	100	>100	>100	>100	>100	2
13	100	100	100	>100	>100	10
14	100	100	100	>100	>100	<1
15	>100	>100	>100	>100	>100	<1
16	>100	>100	>100	>100	>100	100
17	100	>100	>100	>100	>100	256
18	>100	>100	>100	>100	>100	3
19	>100	>100	>100	>100	>100	2
20	>100	>100	>100	>100	>100	51
21	100	>100	100	>100	>100	8
22	>100	>100	>100	>100	>100	45
23	>100	>100	>100	>100	>100	100
24	>100	>100	>100	>100	>100	2
25	>100	>100	>100	>100	>100	42
26	>100	>100	>100	>100	>100	1
27	>100	>100	>100	>100	>100	18
28	>100	>100	>100	>100	>100	1
29	100	>100	100	>100	>100	8
30	100	>100	>100	>100	>100	191
31	>100	>100	>100	>100	>100	1
32	100	100	50	>100	>100	6
33	100	100	100	>100	>100	325

a) MIC was estimated by the agar dilution method. SA: *Staphylococcus aureus* ATCC 6538P. BS: *Bacillus subtilis* ATCC 6633. BC: *Bacillus cereus* IFO3001. EC: *Escherichia coli* NIHJ. KP: *Klebsialla pneumoniae* ATCC 10031. *b*) GMS activity *in vivo* was estimated by means of the 2 × 2 point parallel line assay. The activity of EM-A was taken to be 1.

series of strong contractions were induced in the gastric body and antrum and the contractions migrated along the small intestine in a caudal direction, as shown in Fig. 1. The contractile pattern induced by **3** was quite similar to that of the natural interdigestive migrating contractions and that of synthetic motilin-induced contractions.[5]

The *in vitro* study, moreover, indicated that **3** caused contractions of the rabbit duodenum at a concentration of 10^{-9} M (the minimum effective concentration), which is comparable to the potency of synthetic motilin.[5] The contractile pattern induced by **3**, as shown in Fig. 2, was quite similar to that caused by synthetic motilin, and was quite different from that caused by acetylcholine. The contractions induced by **3** were not blocked by pretreatment with tetrodotoxin (10^{-6} M) or atropine (10^{-6} M), as was also the case with synthetic motilin.

Moreover, we have clarified that EM-A and some derivatives obtained in this study are agonists of motilin at the motilin receptor.[6] Based on these findings, we propose the name "motilides" to describe a series of compounds with macrolide structure and motilin-like activity.

Table V. Antimicrobial Activity (MIC) and GMS Activity in the Dog (*in Vivo*) of Quaternary Ammonium Derivatives of **2**

Compound No.	Antimicrobial activity: MIC[a] (μg/ml)					GMS activity[b]
	SA	BS	BC	EC	KP	
EM-A	0.2	0.1	0.1	12.5	6.25	1
2	12.5	6.25	12.5	>100	>100	2
36	>100	>100	>100	>100	>100	65
37	>100	>100	>100	>100	>100	30
38	>100	>100	>100	>100	>100	3
39	100	50	100	>100	>100	13
40	100	100	100	>100	>100	115
41	>100	>100	>100	>100	>100	202
42	25	25	25	>100	>100	<1
43	25	25	12.5	>100	>100	69
44	100	100	100	>100	>100	24
45	>100	>100	>100	>100	>100	11
46	50	50	100	>100	>100	5
47	50	50	50	>100	>100	1

a, b) See footnote *a* and *b* of Table IV.

Motilides may be useful not only to modulate the contractile activity in the gastrointestinal tract but also to study the physiology and control mechanisms of gastrointestinal motility.

Experimental

NMR spectra were measured on either a JEOL FX-90Q or Varian XL-400 spectrometer in $CDCl_3$ or $CDOD_3$ solution. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard. FAB-mass spectra were obtained on JEOL D-100 and DX-300 spectrometers. Elemental analysis for carbon, hydrogen and nitrogen was done with a Perkin-Elmer model 240 elemental analyzer and were within ±0.4% of theoretical values. Optical rotations were measured with a Jasco DIP-181 polarimeter. Silica gel column chromatography was performed with Merck Kieselgel 60. All starting materials were obtained commercially unless otherwise indicated.

***N*-Propargyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (3) (Method A)** Propargyl bromide (0.9 ml, 10.1 mmol) was added to a stirred solution of **1** (1.0 g, 1.4 mmol) in $CHCl_3$ (10 ml) and the reaction mixture was stirred at ambient temperature for 6 h. The reaction mixture was concentrated *in vacuo* and the residue was taken up in Et_2O (20 ml). The resulting crude product was filtered off, washed with Et_2O (20 ml × 2) and purified by column chromatography on silica gel, eluting with $CHCl_3$–MeOH–concentrated NH_4OH (10 : 1 : 0.1), to give **3** as a white amorphous solid: 550 mg (48%).

***N*-Methyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (4)** CH_3I (40 μl, 0.64 mmol) was added to a stirred solution of **1** (100 mg, 0.14 mmol) in $CHCl_3$ (1 ml) and the reaction mixture was stirred at ambient temperatue for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was taken up in Et_2O (5 ml). The resulting crude product was filtered off and washed with Et_2O (5 ml × 2) to give **4** as a white amorphous solid: 65 mg (54%).

***N*-Ethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (5)** A solution of **1** (200 mg, 0.28 mmol) and ethyl iodide (0.5 ml, 6.3 mmol) in $CHCl_3$ (4 ml) was stirred at 60 °C for 20 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **5** as a white amorphous solid: 145 mg (60%).

***N*-Propyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (6)** A solution of **1** (200 mg, 0.28 mmol) and propyl iodide (0.5 ml, 5 mmol) in $CHCl_3$ (4 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **6** as a white amorphous solid: 120 mg (48%).

***N*-Butyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (7)** A solution of **1** (150 mg, 0.21 mmol) and butyl iodide (1.0 ml, 8.8 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 80 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **7** as a white amorphous solid: 121 mg (64%).

***N*-Benzyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Chloride (8)** A solution of **1** (200 mg, 0.28 mmol) and benzyl chloride (0.3 ml, 2.6 mmol) in

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TABLE VI. ^{13}C-NMR Chemical Shifts (δ) of the Derivatives

Carbon No.	3	4	5	6	7	8	16	17	18	19	9	10	11	12	13
1	179.1	179.2	179.2	179.2	179.2	179.2	179.0	179.0	179.0	178.9	179.0	179.1	179.1	179.1	179.0
2	46.0	46.1	46.2	46.0	46.1	46.2	45.9	46.0	45.9	45.8	46.0	46.0	46.0	46.1	46.0
3	78.5	78.6	78.6	78.6	78.6	78.4	78.4	78.5	78.5	78.2	78.5	78.5	78.5	78.5	78.4
4	44.6	44.7	44.6	44.6	44.7	44.8	44.6	44.6	44.5	44.5	44.5	44.5	44.7	44.7	44.6
5	82.3	82.4	82.3	82.3	82.3	82.1	82.0	82.1	81.9	82.0	82.2	82.4	82.1	82.3	82.1
6	86.5	86.6	86.6	86.5	86.6	86.6	86.4	86.5	86.4	86.4	86.4	86.4	86.6	86.6	86.5
7	43.5	44.2	43.4	43.4	43.4	43.5	43.4	43.4	43.4	43.4	43.4	43.4	43.5	43.5	43.5
8	102.4	102.5	102.4	102.4	102.5	102.5	102.3	102.3	102.3	102.1	102.3	102.4	102.4	102.4	102.4
9	153.6	153.5	153.6	153.5	153.5	153.7	153.4	153.4	153.4	153.5	153.4	153.5	153.5	153.6	153.4
10	32.4	32.4	32.3	32.3	32.3	32.4	32.2	32.3	32.2	32.3	32.2	32.3	32.4	32.4	32.3
11	71.2	71.3	71.1	71.2	71.2	71.3	71.2	71.2	71.2	71.0	71.2	71.2	71.1	71.3	71.1
12	76.6	76.6	76.6	76.6	76.6	76.6	76.5	76.5	76.5	76.4	76.5	76.5	76.6	76.7	76.5
13	79.1	79.1	79.0	79.0	79.1	79.1	78.9	79.0	78.9	78.9	78.9	79.0	79.1	79.2	79.0
14	21.6	21.7	21.7	21.6	21.7	21.7	21.6	21.7	21.6	21.6	21.6	21.6	21.8	21.8	21.8
15	11.2	11.3	11.2	11.2	11.3	11.3	11.2	11.2	11.2	11.2	11.2	11.3	11.3	11.3	11.3
16	14.1	14.2	14.2	14.2	14.3	14.2	14.2	14.2	14.1	14.0	14.2	14.2	14.2	14.2	14.2
17	9.3	9.4	9.4	9.4	9.5	9.5	9.4	9.4	9.4	9.3	9.3	9.4	9.4	9.4	9.4
18	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.8	26.8	26.8	26.8	26.9	26.9	26.9	26.9
19	12.2	12.3	12.2	12.2	12.3	12.3	12.3	12.2	12.3	12.3	12.2	12.3	12.3	12.3	12.3
20	17.3	17.4	17.3	17.3	17.4	17.4	17.4	17.4	17.4	17.3	17.4	17.4	17.4	17.4	17.4
21	16.0	16.2	16.0	16.1	16.2	16.2	16.1	16.1	16.2	16.1	16.1	16.2	16.2	16.2	16.2
1′	103.0	103.1	103.1	103.1	103.1	103.2	103.0	103.0	103.2	103.2	103.0	102.8	103.1	103.2	103.1
2′	72.9	74.2	72.6	72.6	72.6	73.2	73.4	72.7	72.7	72.8	73.3	74.1	72.8	72.9	72.7
3′	68.0	67.8	67.7	67.7	67.8	68.0	67.7	67.7	67.9	67.9	67.7	67.7	67.9	67.8	67.8
4′	34.4	34.6	34.2	34.2	34.2	34.4	34.2	34.2	34.2	34.2	34.1	34.2	34.3	34.4	34.2
5′	72.2	72.9	71.5	71.9	71.8	72.1	72.6	72.7	71.2	71.0	72.6	72.6	72.3	72.9	71.5
6′	21.6	21.7	21.7	21.6	21.7	21.7	21.6	21.7	21.6	21.6	21.6	21.6	21.8	21.7	21.8
3′-NCH_3	49.0	54.7	49.0	49.0	49.1	49.1	49.0	49.0	49.0	49.0	49.0	49.1	49.1	49.1	49.1
1″	96.6	96.6	96.5	96.6	96.6	96.6	96.4	96.5	96.3	96.3	96.5	96.5	96.6	96.6	96.4
2″	35.8	35.8	35.7	35.7	35.7	35.9	35.7	35.7	35.7	35.7	35.7	35.7	35.8	35.8	35.7
3″	74.6	74.6	74.7	74.6	74.6	74.7	74.4	74.5	74.4	74.3	74.5	74.6	74.6	74.7	74.6
4″	79.1	79.1	79.0	79.0	79.1	79.1	78.9	79.0	79.2	79.3	78.9	79.0	79.1	79.1	79.0
5″	66.7	66.8	66.7	66.7	66.8	66.7	66.6	66.6	66.6	66.6	66.6	66.7	66.7	66.7	66.7
6″	18.9	18.9	18.9	18.9	19.0	19.0	18.9	18.9	19.0	18.8	18.9	19.0	19.0	19.0	19.0
7″	22.1	22.1	22.1	22.1	21.9	22.1	21.8	22.1	21.8	22.1	21.7	23.0	22.2	22.2	22.1
3″-OCH_3	50.2	50.2	50.1	50.0	50.1	50.3	50.0	50.1	50.0	49.9	49.9	50.3	50.1	50.0	50.1
	57.6		62.2	68.1	66.8	69.7	57.0	66.6	66.3	67.9	73.3	55.0	71.1	68.7	68.7
	73.2		8.8	17.3	25.8	131.8	67.4	129.2	169.5	169.2	82.2	112.7	5.4	18.6	142.3
	83.2			10.8	20.8	130.2		127.0	19.0				5.9	142.4	119.7
					14.1	134.8								119.7	18.6
						129.4									

$CHCl_3$ (4 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **8** as a white amorphous solid: 122 mg (52%).

***N*-2-Fluoroethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (9)** A solution of **1** (150 mg, 0.21 mmol) and 1-bromo-2-fluoroethane (0.5 ml, 6.7 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 120 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **9** as a white amorphous solid: 135 mg (76%).

***N*-Cyanomethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (10)** A solution of **1** (150 mg, 0.21 mmol) and bromoacetonitrile (0.5 ml, 7.2 mmol) in $CHCl_3$ (1 ml) was stirred at ambient temperature for 5 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **10** as a white amorphous solid: 165 mg (94%).

***N*-Cyclopropylmethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (11)** A solution of **1** (150 mg, 0.21 mmol) and cyclopropylmethyl bromide (0.3 ml, 3.1 mmol) in $CHCl_3$ (2 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **11** as a white amorphous solid: 145 mg (81%).

***N*-3-Butenyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (12)** A solution of **1** (150 mg, 0.21 mmol) and 4-brom-1-butene (0.5 ml, 4.9 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 18 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **12** as a white amorphous solid: 91 mg (56%).

***N*-Crotyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (13)** A solution of **1** (150 mg, 0.21 mmol) and crotyl bromide (0.5 ml, 3.1 mmol) in $CHCl_3$ (2 ml) was stirred at ambient temperature for 6 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **13** as a white amorphous solid: 175 mg (98%).

***N*-2-Bromo-2-propenyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (14)** A solution of **1** (150 mg, 0.21 mmol) and 2,3-dibromo-1-propene (0.5 ml, 5.3 mmol) in $CHCl_3$ (1.5 ml) was stirred at ambient temperature for 24 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **14** as a white amorphous solid: 111 mg (58%).

***N*-2-Butynyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (15)** A solution of **1** (150 mg, 0.21 mmol) and 1-bromo-2-butyne (0.3 ml, 2.3 mmol) in $CHCl_3$ (2 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **15** as a white amorphous solid: 162 mg (100%).

***N*-2-Hydroxyethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (16) (Method B)** A solution of **1** (120 mg, 0.17 mmol), *N*,*N*-diisopropylethylamine (0.5 ml, 2.9 mmol) and 2-bromoethanol (0.5 ml, 7.1 mmol) in $CHCl_3$ (1 ml) was stirred at reflux for 48 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **16** as a white amorphous solid: 119 mg (84%).

***N*-Allyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (17)** A solution of **1** (150 mg, 0.21 mmol), *N*,*N*-diisopropylethylamine (0.25 ml, 1.5 mmol) and allyl bromide (0.5 ml, 5.8 mmol) in $CHCl_3$ (1 ml) was stirred at reflux for 24 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **17** as a white amorphous solid: 134 mg (76%).

***N*-Methoxycarbonylmethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (18)** A solution of **1** (150 mg, 0.21 mmol), *N*,*N*-diisopropyl-

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TABLE VI. (continued)

Carbon No.	14	15	20	21	22	23	24	25	26	27	28	29	30	31
1	179.2	179.0	179.2	179.2	179.2	179.2	179.1	179.1	179.2	179.1	179.1	179.1	179.1	179.2
2	46.1	46.0	45.8	46.1	46.1	46.1	46.1	46.2	46.1	45.7	46.1	46.0	46.0	46.1
3	78.6	78.4	78.7	78.6	78.6	78.6	78.5	78.6	78.6	78.6	78.6	78.4	78.4	78.4
4	44.8	44.6	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.7	44.6	44.7
5	82.3	82.2	82.5	82.3	82.4	82.2	82.3	82.4	82.4	82.4	82.3	82.0	82.2	82.0
6	86.6	86.5	86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.5	86.7
7	43.5	43.4	43.6	43.6	43.5	43.5	43.5	43.5	43.5	43.5	43.5	43.5	43.5	43.5
8	102.5	102.4	102.5	102.5	102.5	102.4	102.4	102.4	102.4	102.4	102.4	102.4	102.4	102.4
9	153.7	153.5	153.7	153.7	153.5	153.6	153.7	153.6	153.7	153.7	153.7	153.6	153.6	153.7
10	32.4	32.3	32.4	32.4	32.3	32.4	32.4	32.4	32.4	32.4	32.4	32.4	32.4	32.4
11	71.3	71.1	71.3	71.3	71.1	71.2	71.2	71.2	71.2	71.2	71.2	71.2	71.2	71.2
12	76.7	76.5	76.7	76.7	76.6	76.6	76.6	76.6	76.6	76.6	76.6	76.6	76.6	76.5
13	79.2	79.0	79.1	79.2	79.0	79.0	79.0	79.1	79.1	79.1	79.1	79.0	79.1	79.1
14	21.8	21.7	21.7	21.8	21.7	21.7	21.6	21.7	21.6	21.7	21.7	21.6	21.7	21.6
15	11.3	11.3	11.2	11.3	11.3	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2
16	14.3	14.2	14.2	14.2	14.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3
17	9.4	9.5	9.5	9.5	9.5	9.5	9.6	9.4	9.4	9.5	9.5	9.3	9.3	9.3
18	27.0	26.9	27.0	27.0	27.0	26.9	26.9	27.0	27.1	26.9	26.9	26.8	26.9	26.8
19	12.3	12.3	12.3	12.3	12.4	14.2	14.2	14.2	14.2	14.2	14.2	14.1	14.0	14.1
20	17.5	17.4	17.4	17.4	17.4	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2
21	16.2	16.2	16.1	16.1	16.2	16.0	16.1	16.1	16.1	16.0	16.0	16.0	16.0	16.0
1′	103.1	102.9	103.2	103.2	103.4	103.3	103.6	103.2	103.6	103.7	103.4	103.2	103.0	103.4
2′	73.0	72.8	73.1	72.9	73.4	72.5	72.9	75.2	72.7	72.8	72.5	72.8	72.9	73.2
3′	68.0	67.8	68.0	67.9	67.7	67.8	68.3	67.9	68.1	67.7	68.0	67.9	68.0	68.4
4′	34.3	34.4	34.3	34.4	34.1	34.1	35.9	35.4	35.8	33.3	33.9	34.3	34.8	35.7
5′	73.0	72.8	70.8	72.9	72.1	70.1	70.0	72.1	72.7	70.1	68.5	70.6	72.7	70.5
6′	21.8	21.7	21.7	21.8	21.7	22.1	22.1	22.1	22.1	22.1	22.1	22.1	22.2	22.1
3′-NCH_3	49.1	49.1	47.2	49.1	49.1	47.1	47.1	42.9		48.0		47.1	47.1	
1″	96.6	96.5	96.7	96.7	96.6	96.6	96.6	96.6	96.6	96.6	96.6	96.6	96.6	96.6
2″	35.9	35.8	35.8	35.8	35.8	35.8	35.7	35.8	35.8	35.8	35.8	35.8	35.8	35.7
3″	74.7	74.6	74.8	74.7	74.6	74.7	74.7	74.7	74.7	74.7	74.7	74.6	74.7	74.6
4″	79.2	79.0	79.1	79.2	79.0	79.0	79.0	79.1	79.1	79.0	79.0	79.0	79.1	79.1
5″	66.8	66.7	66.8	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7
6″	19.0	19.0	19.0	19.0	19.0	19.0	19.0	18.9	18.9	18.9	18.9	18.9	18.9	18.9
7″	22.2	22.1	22.2	22.2	21.7	21.7	21.6	21.6	21.6	21.7	21.7	21.6	21.7	21.6
3″-OCH_3	50.1	50.0	50.0	50.0	50.1	50.0	50.0	50.2	50.0	50.0	50.0	49.9	50.3	50.0
	66.3	58.5	67.8	74.3	64.5	57.8	55.2	69.7	65.2	64.9	59.8	66.0	53.9	63.2
	135.7	68.8	16.1	24.8	18.1	57.1	9.6	65.3	62.1	62.1	57.2	64.2	53.2	128.3
	119.7	90.5	16.9	23.6	72.6	8.8	9.4	22.8	23.0	21.3	21.1	129.1	83.8	127.5
	3.7			23.6	79.5	8.6		20.5	22.4	21.2	20.8	127.0	83.7	
									55.7	22.1	22.1	126.8	72.5	
									10.3		53.4		72.1	

ethylamine (0.5 ml, 2.9 mmol) and methyl bromoacetate (1 ml, 10.6 mmol) in $CHCl_3$ (1 ml) was stirred at reflux for 6 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **18** as a white amorphous solid: 145 mg (80%).

***N*-Carboxymethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (19)** A solution of **1** (150 mg, 0.21 mmol), *N*,*N*-diisopropylethylamine (0.5 ml, 0.29 mmol) and bromoacetic acid (200 mg, 1.4 mmol) in $CHCl_3$ (1 ml) was stirred at reflux for 6 h. The reaction mixture was worked up in a similar manner to that described for the preparation of **4** to give **19** as a white amorphous solid: 127 mg (71%).

***N*-Isopropyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (20) (Method C)** A solution of de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (50 mg, 0.07 mmol) and CH_3I (0.1 ml, 1.6 mmol) in $CHCl_3$ (0.5 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up as described for the preparation of **4** to give **20** as a white amorphous solid: 52 mg (86%).

***N*-Isobutyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (21)** A solution of de(*N*-methyl)-*N*-isobutyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (29 mg, 0.04 mmol) and CH_3I (0.4 ml, 6.4 mmol) in $CHCl_3$ (0.3 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up as described for the preparation of **4** to give **21** as a white amorphous solid: 30 mg (86%).

***N*-3-Butynyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (22)** A solution of de(*N*-methyl)-*N*-3-butynyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (78 mg, 0.1 mmol) and CH_3I (0.5 ml, 8 mmol) in $CHCl_3$ (0.8 ml) was stirred at 40 °C for 9 h. The reaction mixture was worked up as described for the preparation of **4** to give **22** as a white amorphous solid: 85 mg (92%).

De(*N*-methyl)-*N*,*N*-diethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (23) A solution of de(*N*-methyl)-*N*-ethyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (88 mg, 0.12 mmol) and ethyl iodide (1 ml, 12.6 mmol) in $CHCl_3$ (2 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **23** as a white amorphous solid: 72 mg (67%). *Anal.* Calcd for $C_{40}H_{72}INO_{12}$: C, 54.23; H, 8.19; N, 1.58. Found: C, 54.43; H, 7.92; N, 1.74.

Bis-de(*N*-methyl)-*N*,*N*,*N*-triethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (24) A solution of bis-de(*N*-methyl)-*N*,*N*-diethyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (88 mg, 0.12 mmol) and ethyl iodide (1 ml, 12.6 mmol) in $CHCl_3$ (2 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **24** as a white amorphous solid: 15 mg (16%). *Anal.* Calcd for $C_{41}H_{74}INO_{12}$: C, 54.72; H, 8.29; N, 1.56. Found: C, 54.83; H, 8.02; N, 1.76.

De(dimethylamino)-3′-*N*-methylpyrrolidino-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (25) A solution of de(dimethylamino)-3′-pyrrolidino-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (63 mg, 0.09 mmol) and CH_3I (0.1 ml, 1.6 mmol) in $CHCl_3$ (2 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up as described for the preparation of **4** to give **25** as a white amorphous solid: 70 mg (93%). *Anal.* Calcd for $C_{40}H_{70}INO_{12}$: C, 54.36; H, 7.98; N, 1.59. Found: C, 54.53; H, 7.65; N, 1.74.

De(dimethylamino)-3′-*N*-ethylpyrrolidino-8,9-anhydroerythromycin A

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TABLE VI. (continued)

Carbon No.	32	33	36	37	38	39	40	41	42	43	44	45	46	47
1	179.1	179.1	179.6	179.6	179.6	179.6	179.5	179.5	179.6	179.6	179.7	179.5	179.6	179.6
2	46.1	45.9	46.6	46.6	46.6	46.6	46.6	46.5	46.6	46.5	46.7	46.5	46.5	46.5
3	78.2	78.2	78.3	78.4	78.3	78.4	78.3	78.1	78.3	78.3	78.5	78.1	78.2	78.2
4	44.6	44.6	45.3	45.3	45.3	45.3	45.3	45.3	45.5	45.2	45.4	45.2	45.2	45.2
5	82.0	82.2	85.5	85.5	85.6	85.5	85.5	85.5	85.1	85.4	85.6	85.2	85.5	85.5
6	86.7	86.5	85.5	85.5	85.6	85.5	85.5	85.5	85.6	85.4	85.6	85.5	85.5	85.5
7	43.5	43.5	42.0	42.2	42.1	42.1	42.0	42.0	42.0	42.2	42.0	41.9	42.1	42.1
8	102.4	102.4	37.4	37.5	37.4	37.4	37.4	37.4	37.5	37.4	37.5	37.4	37.4	37.4
9	153.6	153.6	85.5	85.6	85.6	85.5	85.5	85.5	85.6	85.4	85.6	85.5	85.5	85.5
10	32.4	32.3	35.2	35.2	35.2	35.2	35.2	35.1	35.2	35.2	35.2	35.0	35.2	35.2
11	71.3	71.2	74.2	74.3	74.2	74.2	74.2	74.2	74.3	74.4	74.2	74.3	74.3	74.5
12	76.6	76.6	75.8	75.8	75.7	75.5	75.7	75.7	75.9	75.7	75.9	75.8	75.7	75.7
13	79.1	79.1	80.2	80.3	80.2	80.2	80.2	80.2	80.3	80.2	80.3	80.2	80.3	80.3
14	21.6	21.7	21.8	21.8	21.8	21.8	21.7	21.8	21.7	21.7	21.8	21.8	21.7	21.8
15	11.2	11.2	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4
16	17.3	17.3	14.4	14.4	14.4	14.5	14.4	14.3	14.4	14.4	14.5	14.3	14.3	14.3
17	9.3	9.3	10.6	10.8	10.8	10.8	10.6	10.6	10.4	10.7	10.5	10.6	10.7	10.6
18	26.8	26.8	30.1	30.1	30.1	30.1	30.1	30.1	30.3	30.1	30.3	30.1	30.1	30.1
19	14.0	14.0	17.9	18.0	17.9	18.0	17.9	17.9	17.9	17.9	17.9	17.9	17.9	18.0
20	12.2	12.2	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4
21	16.0	16.0	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.7	18.7	18.8
1′	103.3	103.2	103.1	103.2	103.2	103.2	103.2	103.0	103.0	103.3	103.3	103.2	103.2	103.1
2′	73.1	73.0	74.2	74.3	74.2	74.2	74.2	74.2	74.3	74.4	74.2	74.3	74.3	74.5
3′	68.3	68.4	67.9	68.0	68.0	67.9	68.0	68.0	68.1	68.4	67.9	67.8	68.0	68.0
4′	35.7	36.1	34.5	34.2	34.2	34.2	34.2	34.3	34.3	34.2	34.2	34.1	34.2	34.3
5′	71.3	72.3	72.8	72.5	72.5	72.5	72.7	72.6	73.2	72.6	72.8	72.7	72.6	72.6
6′	21.6	22.1	21.8	21.8	21.8	21.8	21.7	21.8	21.7	21.7	21.8	21.8	21.7	21.8
3′-NCH_3			53.7	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0
1″	96.5	96.5	96.1	96.2	96.1	96.1	96.1	96.1	96.2	96.2	96.2	96.1	96.2	96.1
2″	35.7	35.8	35.8	35.8	35.8	35.8	35.8	35.8	35.9	35.8	35.8	35.8	35.8	35.8
3″	74.6	74.6	74.5	74.6	74.5	74.5	74.5	74.5	74.6	74.4	74.7	74.3	74.3	74.5
4″	79.1	79.1	79.1	79.2	79.1	79.1	79.1	79.2	79.2	79.2	79.1	79.1	79.1	79.1
5″	66.7	66.7	66.4	66.5	66.4	66.4	66.4	66.4	66.5	66.5	66.6	66.4	66.4	66.4
6″	18.9	18.9	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.7	18.7	18.7
7″	21.6	21.7	22.5	22.5	22.5	22.5	22.5	22.5	22.6	22.5	22.5	22.4	22.5	22.5
3″-OCH_3	50.0	50.3	49.9	50.0	50.1	50.1	50.1	50.2	50.0	50.0	50.0	49.9	49.9	49.0
	63.2	51.7		62.2	68.0	66.7	66.6	57.6	69.8	57.0	73.5	55.0	71.0	18.5
	129.2	84.3		8.7	17.2	25.7	129.3	73.1	130.3	67.1	82.6	112.7	5.2	68.6
	127.0	72.3			11.0	20.7	127.0	83.1	131.8				5.7	119.5
						14.0			134.8					142.3
	50.9													
	84.0													
	73.1													

6,9-Hemiacetal Iodide (26) A solution of de(dimethylamino)-3′-pyrrolidino-8,9-anhydroerythromycin A, 6,9-hemiacetal[2] (93 mg, 0.13 mmol) and ethyl iodide (1 ml, 12.6 mmol) in $CHCl_3$ (2 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **26** as a white anirogiys solid: 94 mg (84%). *Anal.* Calcd for $C_{41}H_{72}INO_{12}$: C, 54.84; H, 8.08; N, 1.56. Found: C, 54.56; H, 7.84; N, 1.84.

De(dimethylamino)-3′-*N*-methylpiperidino-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (27) A solution of de(dimethylamino)-3′-piperidino-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (83 mg, 0.11 mmol) and CH_3I (0.5 ml, 8.0 mmol) in $CHCl_3$ (0.5 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up as described for the preparation of **4** to give **27** as a white amorphous solid: 84 mg (85%).

De(dimethylamino)-3′-*N*-ethylpiperidino-8,9-anhydroerythromycin A 6,9-Hemiacetal Iodide (28) A solution of de(dimethylamino)-3′-piperidino-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (94 mg, 0.12 mmol) and ethyl iodide (1 ml, 12.6 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **28** as a white amorphous solid: 33 mg (29%).

De(*N*-methyl)-*N*,*N*-diallyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (29) A solution of de(*N*-methyl)-*N*-allyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (100 mg, 0.15 mmol) and allyl bromide (0.1 ml, 1.2 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 14th. The reaction mixture was worked up as described for the preparation of **4** to give **29** as a white amorphous solid: 110 mg (94%).

De(*N*-methyl)-*N*,*N*-dipropargyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (30) A solution of de(*N*-methyl)-*N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (61 mg, 0.08 mmol) and propargyl bromide (0.1 ml, 1.1 mmol) in $CHCl_3$ (0.5 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **30** as a white amorphous solid: 51 mg (72%).

Bis-de(*N*-methyl)-*N*,*N*,*N*-triallyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (31) A solution of bis-de(*N*-methyl)-*N*,*N*-diallyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (99 mg, 0.13 mmol) and allyl bromide (0.1 ml, 1.2 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 14 h. The reaction mixture was worked up as described for the preparation of **4** to give **31** as a white amorphous solid: 16 mg (14%).

Bis-de(*N*-methyl)-*N*,*N*-diallyl-*N*-propargyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (32) A solution of bis-de(*N*-methyl)-*N*,*N*-diallyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (61 mg, 0.08 mmol), $NaHCO_3$ (12 mg, 0.14 mmol) and propargyl bromide (82 μl, 0.9 mmol) in MeOH (1 ml) was stirred at ambient temperature for 3 d. The reaction mixture was worked up as described for the preparation of **4** to give **32** as a white amorphous solid: 32 mg (39%).

Bis-de(*N*-methyl)-*N*,*N*,*N*-tripropargyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (33) A solution of bis-de(*N*-methyl)-*N*,*N*-dipropar-

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gyl-8,9-anhydroerythromycin A 6,9-hemiacetal[2] (101 mg, 0.13 mmol), $NaHCO_3$ (24 mg, 0.28 mmol) and propargyl bromide (0.1 ml, 1.1 mmol) in MeOH (2 ml) was stirred at ambient temperature for 3 d. The reaction mixture was worked up as described for the preparation of **4** to give **33** as a white amorphous solid: 38 mg (30%).

***N*-Ethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Bromide (34)** A solution of **1** (200 mg, 0.28 mmol), and ethyl bromide (0.5 ml, 6.7 mmol) in $CHCl_3$ (4 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up as described for the preparation of **4** to give **34** as a white amorphous solid: 189 mg (82%).

***N*-Ethyl-8,9-anhydroerythromycin A 6,9-Hemiacetal Acetate (35)** A solution of **5** (50 mg, 0.06 mmol), acetic acid (0.1 ml) and silver oxide (100 mg, 0.43 mmol) in 50% MeOH (8 ml) was stirred vigorously for 1 d. The reaction mixture was filtered and concentrated *in vacuo*. The resulting crude product was purified by column chromatography on silica gel, eluting with $CHCl_3$–MeOH–concentrated NH_4OH (10 : 1 : 0.1), to give **35** as a white amorphous solid: 25 mg (53%). *Anal.* Calcd for $C_{41}H_{73}NO_{14}$: C, 61.25; H, 9.15; N, 1.74. Found: C, 60.98, H, 8.86; N, 1.95.

***N*-Methyl-9,9-dihydroerythromycin A 6,9-Epoxide Iodide (36)** A solution of **2** (100 mg, 0.14 mmol) and CH_3I (0.6 ml, 9.6 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 1.5 h. The reaction mixture was worked up as described for the preparation of **4** to give **36** as a white amorphous solid: 85 mg (71%).

***N*-Ethyl-9,9-dihydroerythromycin A 6,9-Epoxide Iodide (37)** A solution of **2** (100 mg, 0.14 mmol) and ethyl iodide (0.6 ml, 7.6 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up as described for the preparation of **4** to give **37** as a white amorphous solid: 90 mg (74%).

***N*-Propyl-9,9-dihydroerythromycin A 6,9-Epoxide Iodide (38)** A solution of **2** (100 mg, 0.14 mmol) and propyl iodide (0.7 ml, 7 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 48 h. The reaction mixture was worked up as described for the preparation of **4** to give **38** as a white amorphous solid: 87 mg (70%).

***N*-Butyl-9,9-dihydroerythromycin A 6,9-Epoxide Iodide (39)** A solution of **2** (100 mg, 0.14 mmol) and butyl iodide (1.0 ml, 8.8 mmol) in $CHCl_3$ (1 ml) was stirred at 60 °C for 80 h. The reaction mixture was worked up as described for the preparation of **4** to give **39** as a white amorphous solid: 95 mg (76%).

***N*-Allyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (40)** A solution of **2** (200 mg, 0.28 mmol) and allyl bromide (0.5 ml, 5.8 mmol) in $CHCl_3$ (2 ml) was stirred at 60 °C for 24 h. The reaction mixture was worked up as described for the preparation of **4** to give **40** as a white amorphous solid: 190 mg (90%).

***N*-Propargyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (41)** A solution of **2** (200 mg, 0.28 mmol) and propargyl bromide (0.5 ml, 5.6 mmol) in $CHCl_3$ (2 ml) was stirred at ambient temperature for 2 h. The reaction mixture was worked up as described for the preparation of **4** to give **41** as a white amorphous solid: 155 mg (81%).

***N*-Benzyl-9,9-dihydroerythromycin A 6,9-Epoxide Chloride (42)** A solution of **2** (150 mg, 0.21 mmol) and benzyl chloride (0.5 ml, 4.2 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 38 h. The reaction mixture was worked up as described for the preparation of **4** to give **42** as a white amorphous solid: 155 mg (81%).

***N*-2-Hydroxyethyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (43)** A solution of **2** (150 mg, 0.21 mmol) and 2-bromoethanol (0.5 ml, 7.1 mmol) in $CHCl_3$ (3 ml) was stirred at reflux for 24 h. The reaction mixture was worked up as described for the preparation of **4** to give **43** as a white amorphous solid: 118 mg (67%).

***N*-2-Fluoroethyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (44)** A solution of **2** (150 mg, 0.21 mmol) and 1-bromo-2-fluoroethane (0.5 ml, 6.7 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 168 h. The reaction mixture was worked up as described for the preparation of **4** to give **44** as a white amorphous solid: 66 mg (37%).

***N*-Cyanomethyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (45)** A solution of **2** (150 mg, 0.21 mmol) and bromoacetonitrile (0.5 ml, 7.2 mmol) in $CHCl_3$ (3 ml) was stirred at ambient temperature for 40 min. The reaction mixture was worked up as described for the preparation of **4** to give **45** as a white amorphous solid: 111 mg (64%).

***N*-Cyclopropylmethyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (46)** A solution of **2** (150 mg, 0.21 mmol) and cyclopropylmethyl bromide (0.5 ml, 5.2 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 38 h. The reaction mixture was worked up as described for the preparation of **4** to give **46** as a white amorphous solid: 153 mg (86%).

***N*-3-Butenyl-9,9-dihydroerythromycin A 6,9-Epoxide Bromide (47)** A solution of **2** (150 mg, 0.21 mmol) and 4-bromo-1-butene (0.5 ml, 4.9 mmol) in $CHCl_3$ (3 ml) was stirred at 60 °C for 38 h. The reaction mixture was worked up as deseribed for the preparation of **4** to give **47** as a white amorphous solid: 113 mg (63%).

MIC Determination MIC was estimated by the agar dilution method using heart infusion agar (pH 7.0). The test bacteria were *Staphylococcus aureus* ATCC 6538P (SA), *Bacillus subtilis* ATCC 6633 (BS), *Bacillus cereus* IFO3001 (BC), *Escherichia coli* NIHJ (EC) and *Klebsiella pneumonia* ATCC 10031 (KP).

GMS Activity The GMS activity *in vivo* was measured by means of chronically implanted force transducers on the serosa of the gastrointestinal tract positioned to record circular muscle contraction in the gastric body, the gastric antrum, and the small intestine in fasted conscious dogs. Changes in contractile activity were recorded as contractile waves on a polygraph through amplifiers. Test materials were administered as an intravenous bolus injection at 10—15 min after the termination of the natural interdigestive contractions in the stomach. Quantitative comparisons of GMS activity among the derivatives were made by integrating their contractile waves in the gastric antrum. Relative potency was estimated by the use of a 2×2 parallel line assay with 5 animals at each dose. EM-A served as the reference standard in each assay.

In vitro studies were carried out by using muscle strips (5×20 mm) of the rabbit duodenum. Muscle strips were mounted along their longitudinal axes in organ baths containing 20 ml of Tyrode's solution kept at 37 °C and bubbled continuously with 5% CO_2 and 95% O_2. Isotonic contractions of strips were recorded by means of isotonic transducers, which were preloaded with 1.0 g. A test material was first dissolved in ethanol (1 mg/0.05 ml), and the same amount of lactobionate was added. Then, the solution was diluted to the required concentration with normal saline.

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