H, OCH_2), 3.86-3.56 (m, 2 H, CHO), 3.42 (d, 2 H, J = 4.5 Hz, CH_2O), 1.6-1.16 (m, 2 H, CH_2CH_3), 0.66 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{21}N_5O_2$), C, H, N.

9-[(1-Hydroxy-2(*S*)-butoxy)methyl]adenine (16). The procedure mentioned earlier for debenzylation was applied to compound **15** to give the product in 95% yield; mp 130 °C; $[\alpha]^{25}_D$ -14.59° (c 1.295, EtOH); UV λ_{max} (pH 1.3) 256 nm (ϵ 1.4 × 10^4), λ_{max} (pH 11) 260 (ϵ 1.4 × 10^4), λ_{max} (EtOH) 260 (ϵ 1.25 × 10^4); ^{1}H NMR (DMSO-d_6) δ 8.6, 8.5 (2 s, 2 H, H-8, H-2), 5.7 (s, 2 H, OCH_2N), 3.6-3.3 (m, 3 H, H_2OH, CHO), 1.5-1.2 (m, 2 H, CH_2CH_3), 0.65 (t, 3 H, J = 6.5 Hz, CH_2CH_3); exact mass calcd 237.1226, found 237.1224.

9-[[1-(Benzyloxy)-2(*S*)-butoxy]methyl]hypoxanthine (17). Chloropurine **11** (1.66 g, 4.7 mmol) was dissolved in methanol-water (50 mL, 6 mL) containing NaOH (16 g, 0.4 mol) and the solution was heated to reflux for 2 h. At the end of this period, methanol was removed under diminished pressure and the residue was diluted with water (100 mL), neutralized with acetic acid, and extracted with $CHCl_3$ (60 mL). Concentration of the dry organic phase afforded a solid, which was recrystallized from ethyl acetate, to furnish the product: yield 0.8 g (57.3%); mp 160 °C; $[\alpha]^{25}_D$ -37.86° (c 1.565, EtOH); ^{1}H NMR ($CDCl_3$) δ 13.1 (br s, 1 H, NH), 8.16, 7.9 (2 s, 2 H, H-8, H-2), 7.2 (s, 5 H, Ar), 5.8-5.5 (m, 2 H, OCH_2N), 4.43 (s, 2 H, CH_2O), 3.8-3.5 (m, 1 H, CHO), 3.43 (d, 2 H, J = 4.5 Hz, CH_2O), 1.66-1.26 (m, 2 H, CH_2CH_3), 0.7 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{20}N_4O_3$) C, H, N.

9-[(1-Hydroxy-2(*S*)-butoxy)methyl]hypoxanthine (18). The procedure mentioned earlier for debenzylation was applied to compound **17** to give the product in 91.3% yield: mp 170 °C; $[\alpha]^{25}_D$ +19.6° (c 1.145, H_2O); UV λ_{max} (pH 1.3) 248 nm (ϵ 1.3 × 10^4), λ_{max} (pH 11) 253 (ϵ 1.35 × 10^4), λ_{max} (EtOH) 245 (ϵ 1.22 × 10^4) 273 (ϵ 6.3 × 10^3); ^{1}H NMR (DMSO-d_6) δ 8.25, 8.1 (2 s, 2 H, H-8, H-2), 5.7-5.55 (br s, 2 H, OCH_2N), 3.6-3.5 (m, 1 H, CHO), 3.5-3.35 (m, 2 H, CH_2OH), 1.5-1.2 (m, 2 H, CH_2CH_3), 0.65 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{10}H_{14}N_4O_3$) C, H, N.

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Registry No. **6**, 14618-80-5; **7**, 117039-90-4; **8**, 117039-91-5; **9**, 117067-40-0; **10**, 117039-92-6; **11**, 117039-93-7; **12**, 117039-94-8; **13**, 117067-41-1; **14**, 117039-95-9; **15**, 117039-96-0; **16**, 117039-97-1; **17**, 117039-98-2; **18**, 117039-99-3; cytosine, 71-30-7; 2-amino-6-chloropurine, 10310-21-1; 6-chloropurine, 87-42-3.

Structure-Activity Relationships among Methoctramine-Related Polymethylene Tetraamines. Chain-Length and Substituent Effects on M-2 Muscarinic Receptor Blocking Activity[1]

Carlo Melchiorre,* Wilma Quaglia, Maria T. Picchio, Dario Giardinà, Livio Brasili, and Piero Angeli

Department of Chemical Sciences, University of Camerino, 62032 Camerino (MC), Italy. *Received March 28, 1988*

Several polymethylene tetraamines related to methoctramine (1) were prepared and evaluated for their blocking activity on M-2 muscarinic receptors in guinea pig atria and ileum. It turned out that antimuscarinic potency depends on the following parameters: (a) nature of the substituent on both inner and outer nitrogens and (b) carbon chain length separating the inner nitrogens as well as the inner and outer nitrogens. Optimum activity at cardiac M-2 muscarinic receptors was associated with the chain lengths present in **1**, that is, eight methylenes between the inner nitrogens and six methylenes between the inner and outer nitrogens. With regard to the substituents, replacement of the benzylic moiety of **1** by a 2-furyl or a 5-methyl-2-furyl nucleus resulted in enhanced potency toward cardiac M-2 muscarinic receptors. In fact, furtramine (**18**) and mefurtramine (**19**) proved to be more potent and more selective than **1**. Moreover, N-methylation of the four nitrogens of **1** gave different effects: methylation of the outer nitrogens, giving **22**, caused a significant decrease in activity whereas methylation of the inner nitrogens, yielding **23**, resulted in an increase in activity in both atria and ileum.

Increasing evidence indicates multiple subtypes for muscarinic receptors. A first subdivision of muscarinic receptors into at least two subtypes was advanced on the basis of their affinity for pirenzepine.[2,3] Thus, muscarinic receptors with high affinity for pirenzepine were named M-1 while those with low affinity for pirenzepine were classified as M-2 muscarinic receptors. Recent cloning studies have confirmed this subclassification as the M-1 muscarinic receptor of the cerebral cortex and the M-2 muscarinic receptor of the heart proved to be distinct gene products and to have different amino acid sequences.[4-7] Furthermore, two additional muscarinic receptor subtypes that appear to be different from M-1 and M-2 types have been cloned from rat brain.[7] However, matters were further complicated by the observation that certain antagonists have different affinity for muscarinic receptors previously classified as M-2 types. For example, 4-DAMP[8-10]

(1) Preliminary results were presented at the International Symposium on Muscarinic Cholinergic Mechanisms, Tel Aviv, November 30-December 4, 1986, Abstracts, p 29, and published in: Quaglia, W.; Cassinelli, A.; Melchiorre, C. In *Muscarinic Cholinergic Mechanisms*; Cohen, S., Sokolovsky, M., Eds.; Freund Publishing House Ltd.: London, 1987; p 302.
(2) Hammer, R.; Berrie, C. P.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Nature (London)* **1980**, *283*, 90.
(3) Hammer, R.; Giachetti, A. *Life Sci.* **1982**, *31*, 2991.
(4) Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; Mishina, M.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Kojima, M.; Matsuo, H.; Hirose, T.; Numa, S. *Nature (London)* **1986**, *323*, 411.
(5) Kubo, T.; Maeda, A.; Sugimoto, K.; Akiba, I.; Mikami, A.; Takahashi, H.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Matsuo, H.; Hirose, T.; Numa, S. *FEBS Lett.* **1986**, *209*, 367.
(6) Peralta, E. G.; Winslow, J. W.; Peterson, G. L.; Smith, D. H.; Ashkenazi, A.; Ramachandran, J.; Schimerlik, M. I.; Capon, D. J. *Science (Washington, D.C.)* **1987**, *236*, 600.
(7) Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. *Science (Washington, D.C.)* **1987**, *237*, 527.
(8) Barlow, R. B.; Berry, K. J.; Glenton, P. A. M.; Nikolaou, N. M.; Soh, K. S. *Br. J. Pharmacol.* **1976**, *58*, 613.
(9) Barlow, R. B.; Kitchen, R. *Br. J. Pharmacol.* **1982**, *77*, 549.

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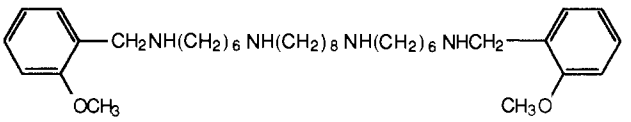
and hexahydrosiladifenidol[11-13] show higher affinity for M-2 muscarinic receptors in smooth muscles than for those in atrium, whereas the reverse is true for AF-DX 116[14-17] and himbacine.[18] The heterogeneity of M-2 muscarinic receptors has been strengthened by the recent discovery of a novel class of antimuscarinic agents which have a polymethylene tetraamine moiety as a basic structural feature and have no structural analogy with previously known antagonists.[19] Polymethylene tetraamines, the prototype being methoctramine (1), have proved to be the most selective antagonists available today toward cardiac M-2 muscarinic receptors in both functional[1,19-25] and binding studies.[22,24-27] Furthermore, methoctramine (1)

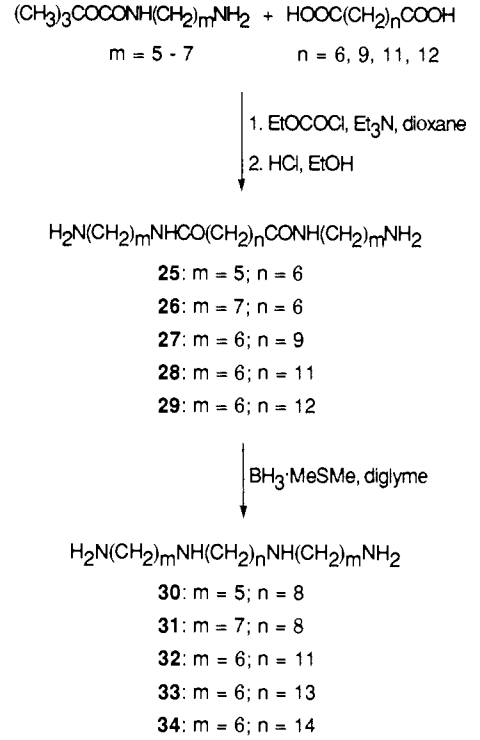


1 (methoctramine)

was also shown to discriminate significantly between M-2 cardiac and M-1 cortical muscarinic receptors in binding assays.[22,26] Thus, polymethylene tetraamines represent a useful tool for muscarinic receptor subclassification in the periphery as well as in the central nervous system. In addition, they may have therapeutic potential in the treatment of synus bradycardia without producing side effects on other parasympathetically innervated organs.

Here we describe the synthesis and in vitro pharmacological properties of several tetraamines related to 1 in order to determine the structural requirements for optimum occupancy of cardiac M-2 muscarinic receptors. Since only tetraamines having five to 10 or 12 methylenes between the inner nitrogens were investigated,[19] we extended the study to compounds **2–4**, bearing 11, 13, and 14 methylenes, respectively. Furthermore, in a previous study[19] the chain length separating the inner from the outer nitrogen was kept constant (six methylenes) owing to the observation that changing it in benextramine[28] gave a decrease in muscarinic activity. Since the optimum chain length between the inner nitrogens was different from that in benextramine,[19] we investigated compounds **5** and **6**, which have the same chain length (eight methylenes) between the inner nitrogens as in **1** but a different one between the inner and outer nitrogens (five or seven instead of six methylenes). In addition, in order to further investigate the effect of substituents on M-2 muscarinic blocking activity, we prepared a series of derivatives carrying benzyl-type substituents (**7–17**) as well as heteroaromatic groups (**18–21**) on the terminal nitrogens of polymethylene tetraamines related to **1**. Finally, compounds **22–24** were included in this study to evaluate the effect of N-methylation of the four secondary amino groups of **1**.

Scheme I

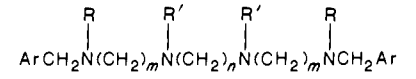


Chemistry. All the compounds were synthesized by standard procedures and were characterized by IR, ^{1}H NMR, and elemental analysis. The structures of the compounds used in the present study are given in Table I.

1,8-Octanedioic acid was amidated with *N*-[(*tert*-butyloxy)carbonyl]-1,5-pentanediamine or *N*-[(*tert*-butyloxy)carbonyl]-1,7-heptanediamine whereas 1,11-undecanedioic acid, 1,13-tridecanedioic acid, and 1,14-tetradecanedioic acid were amidated with *N*-[(*tert*-butyloxy)-carbonyl]-1,6-hexanediamine[29] to give the corresponding intermediate amides. The protecting group was removed by hydrolysis, giving **25–29**, followed by reduction of the amide group to yield unsubstituted tetraamines **30–34**

(10) Barlow, R. B.; Shepherd, M. K. *Br. J. Pharmacol.* **1985**, *85*, 427.
(11) Lambrecht, G.; Moser, U.; Mutschler, E.; Wess, J.; Linoh, H.; Strecker, M.; Tacke, R. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *325* (Suppl.), R25.
(12) Mutschler, E.; Lambrecht, G. *Trends Pharmacol. Sci.* **1984**, *5* (Suppl.), 39.
(13) Mutschler, E.; Moser, U.; Wess, J.; Lambrecht, G. In *Recent Advances in Receptor Chemistry*; Melchiorre, C., Giannella, M., Eds.; Elsevier Science Publishers B.V.: Amsterdam, 1988; p 195.
(14) Hammer, R.; Giraldo, E.; Schiavi, G. B.; Monferini, E.; Ladinsky, H. *Life Sci.* **1986**, *38*, 1653.
(15) Giachetti, A.; Micheletti, R.; Montagna, E. *Life Sci.* **1986**, *38*, 1663.
(16) Micheletti, R.; Montagna, E.; Giachetti, A. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 628.
(17) Giraldo, E.; Monferini, E.; Ladinsky, H.; Hammer, R. *Eur. J. Pharmacol.* **1987**, *141*, 475.
(18) Anwar-ul, S.; Gilani, H.; Cobbin, L. B. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *332*, 16.
(19) Melchiorre, C.; Cassinelli, A.; Quaglia, W. *J. Med. Chem.* **1987**, *30*, 201.
(20) Melchiorre, C.; Angeli, P.; Lambrecht, G.; Mutschler, E.; Picchio, M. T.; Wess, J. *Eur. J. Pharmacol.* **1987**, *144*, 117.
(21) Wess, J.; Lambrecht, G.; Mutschler, E.; Melchiorre, C.; Angeli, P. *Eur. J. Pharmacol.* **1987**, *142*, 475.
(22) Giraldo, E.; Micheletti, R.; Montagna, E.; Giachetti, A.; Viganò, M. A.; Ladinsky, H.; Melchiorre, C. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 1016.
(23) Wess, J.; Angeli, P.; Melchiorre, C.; Moser, U.; Mutschler, E.; Lambrecht, G. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, in press.
(24) Melchiorre, C.; Angeli, P.; Brasili, L.; Giardinâ, D.; Gulini, U.; Pigini, M.; Quaglia, W. In *Actualitês de Chimie Thêrapeutique, 15 Sêries*; Combet Farnoux, C., Ed.; Societê de Chimie Thêrapeutique: Chatenay-Malabry, 1988; p 149.
(25) Melchiorre, C. *Trends Pharmacol. Sci.* **1988**, *9*, 216.
(26) Michel, A. D.; Whiting, R. L. *Eur. J. Pharmacol.* **1988**, *145*, 61.
(27) Michel, A. D.; Whiting, R. L. *Eur. J. Pharmacol.* **1988**, *145*, 305.
(28) Benfey, B. G.; Belleau, B.; Brasili, L.; Giannella, M.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* **1980**, *58*, 1477.
(29) Hansen, J. B.; Nielsen, M. C.; Ehrbar, U.; Buchardt, O. *Synthesis* **1982**, 404.

Table I. pA_2 Values for Polymethylene Tetraamines for Muscarinic Receptors in Guinea Pig Atria and Ileum



no.	Ar	R	R′	*m*	*n*	pA_2[a] (slope) atria	pA_2[a] (slope) ileum	selectivity ratio[b] atria/ileum
1 (methoctramine)	2-$MeOC_6H_4$	H	H	6	8	7.71 ± 0.04 (0.96 ± 0.08)	6.08 ± 0.04 (0.86 ± 0.05)	43
2	2-$MeOC_6H_4$	H	H	6	11	7.43 ± 0.08 (1.21 ± 0.15)	5.64 ± 0.04 (1.14 ± 0.07)	62
3	2-$MeOC_6H_4$	H	H	6	13	7.02 ± 0.14 (1.08 ± 0.13)	5.91 ± 0.04	13
4	2-MeC_6H_4	H	H	6	14	6.87 ± 0.11[c]	5.85 ± 0.04[c]	10
5	2-$MeOC_6H_4$	H	H	5	8	6.79 ± 0.11[c]	5.61 ± 0.08[c]	15
6	2-$MeOC_6H_4$	H	H	7	8	7.56 ± 0.06 (0.86 ± 0.13)	6.19 ± 0.09 (1.06 ± 0.14)	23
7	C_6H_5	H	H	6	8	7.08 ± 0.07 (1.02 ± 0.16)	5.66 ± 0.06 (1.09 ± 0.15)	26
8	3-$MeOC_6H_4$	H	H	6	8	6.82 ± 0.01[c]	5.41 ± 0.08[c]	26
9	4-$MeOC_6H_4$	H	H	6	8	6.85 ± 0.10 (1.02 ± 0.08)	5.56 ± 0.03 (1.09 ± 0.07)	19
10	2-MeC_6H_4	H	H	6	8	7.23 ± 0.07 (0.98 ± 0.17)	5.70 ± 0.01 (1.04 ± 0.09)	34
11	4-MeC_6H_4	H	H	6	8	6.78 ± 0.07[c]	5.63 ± 0.05[c]	14
12	2-ClC_6H_4	H	H	6	8	7.18 ± 0.05 (1.08 ± 0.12)	5.55 ± 0.18[c]	43
13	3-ClC_6H_4	H	H	6	8	6.73 ± 0.06[c]	5.64 ± 0.05[c]	12
14	4-ClC_6H_4	H	H	6	8	6.75 ± 0.10[c]	5.63 ± 0.16[c]	13
15	2-$MeSC_6H_4$	H	H	6	8	7.57 ± 0.06 (1.01 ± 0.07)	6.23 ± 0.04 (1.08 ± 0.11)	22
16	4-$O_2NC_6H_4$	H	H	6	8	7.02 ± 0.09 (1.14 ± 0.25)	5.88 ± 0.16 (1.04 ± 0.19)	14
17	4-FC_6H_4	H	H	6	8	6.89 ± 0.05 (1.11 ± 0.07)	5.62 ± 0.05 (1.10 ± 0.08)	19
18 (furtramine)	2-furyl	H	H	6	8	8.04 ± 0.03 (0.87 ± 0.04)	6.07 ± 0.04 (0.94 ± 0.09)	93
19 (mefurtramine)	5-Me-2-furyl	H	H	6	8	8.05 ± 0.04 (1.11 ± 0.06)	6.15 ± 0.06 (1.00 ± 0.05)	79
20	2-pyridyl	H	H	6	8	7.27 ± 0.16[c]	5.48 ± 0.07[c]	62
21	3-pyridyl	H	H	6	8	6.54 ± 0.07[c]	5.31 ± 0.07[c]	17
22	2-$MeOC_6H_4$	Me	H	6	8	6.78 ± 0.05[c]	<5[d]	>60
23	2-$MeOC_6H_4$	H	Me	6	8	7.99 ± 0.10 (1.14 ± 0.09)	6.38 ± 0.17 (1.25 ± 0.15)	41
24	2-$MeOC_6H_4$	Me	Me	6	8	6.79 ± 0.11[c]	<5[d]	>60
atropine						8.87 ± 0.07 (1.06 ± 0.04)	8.83 ± 0.09 (1.04 ± 0.03)	1

[a] pA_2 and slope values ±SE were calculated from Schild plots,[31] constrained to slope −1.0,[32] unless otherwise specified. pA_2 is the positive value of the intercept of the line derived by plotting log (DR − 1) vs log [antagonist]. The log (DR − 1) was calculated at three different antagonist concentrations, and each concentration was tested at least five times. Dose-ratio (DR) values represent the ratio of the potency of the agonist carbachol (ED_{50}) in the presence of the antagonist and in its absence. Parallelism of dose–response curves was checked by linear regression, and the slopes were tested for significance ($p < 0.05$). [b] The atria/ileum selectivity ratio is the antilog of the difference between the pA_2 values at atria and ileum muscarinic receptors, respectively. [c] Calculated according to van Rossum[33] at only one concentration. [d] Inactive up to a concentration of 10 μM. At higher concentrations dose–response curves to carbachol were shifted to the right with a concomitant depression of the maximum.

(Scheme I). The substituents on the terminal nitrogens were easily introduced by condensation of *N,N′*-bis[6-(amino)hexyl]-1,8-octanediamine[19] and **30–34** with the appropriate aromatic aldehyde and subsequent reduction of the intermediate Schiff bases to give **2–21**.

Condensation of *N,N′*-bis(6-aminocaproyl)-1,8-octanediamine[19] with 2-methoxybenzaldehyde and subsequent reduction of the intermediate Schiff base gave *N,N′*-bis-[6-[(2-methoxybenzyl)amino]caproyl]-1,8-octanediamine. Methylation of the terminal nitrogens followed by reduction of the amide group gave the substituted tetraamine **22**. *N,N′*-Dimethyl analogue **23** was synthesized through amidation of *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid with *N,N′*-dimethyl-1,8-octanediamine[30] followed by removal of the protecting group, reduction of the amide group, and final condensation with 2-methoxybenzaldehyde. The tetramethyl analogue **24** was prepared by methylation of **1**.

Pharmacology. The biological profile of compounds listed in Table I at peripheral muscarinic receptors was assessed by antagonism of carbachol-induced contractions of isolated guinea pig ileum and by antagonism of carbachol-induced inhibition of electrically stimulated guinea pig left atria. In order to allow comparison of the results, atropine and methoctramine (**1**)[19] were used as the standard compounds. The biological results were expressed as pA_2 values determined from Schild plots[31] constrained to slope −1.0,[32] as required by theory. When this method was applied, it was always verified that the experimental data generated a line whose derived slope

(30) Devinsky, F.; Lacko, I.; Krasnec, L. *Synthesis* **1980**, 303.

(31) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.

(32) Tallarida, R. J.; Cowan, A.; Adler, M. W. *Life Sci.* **1979**, *25*, 637.

was not significantly different from unity. However, for compounds with low activity, dissociation constants derived from only one concentration were calculated according to van Rossum.[33]

Results and Discussion

The results assembled in Table I show that all the compounds studied have a significant activity toward muscarinic receptors of guinea pig atria and ileum. With regard to the chain lengths separating the inner nitrogens and the inner from the outer nitrogen, it turned out that optimum activity at cardiac M-2 muscarinic receptors is associated with the same chain lengths as in methoctramine (1). In fact, changing the chain length between inner and outer nitrogens from six methylenes (as in 1) to five and seven methylenes (as in 5 and 6, respectively) gave a decrease in activity in agreement with the results obtained from a similar structural modification performed on benextramine,[28] the prototype of a class of irreversible α-adrenoreceptor antagonists also having muscarinic blocking activity, which represented the starting point for the development of this class of antimuscarinic agents.[19] Similarly, lengthening the distance between the inner nitrogens of 1 from eight to 11, 13, and 14 resulted in a significant decrease in activity.

Substitution of the benzylic moiety of 1 did not improve the activity at cardiac M-2 muscarinic receptors, revealing a special role of the methoxy function of 1 in the interaction with the receptor.

Among the substituents investigated, the furan nucleus gave both qualitative and quantitative improvement of the cardiac M-2 muscarinic receptor blocking profile of 1. In fact, furtramine (18) and mefurtramine (19) proved to be more potent and more selective than 1 toward cardiac M-2 muscarinic receptors. This finding may be relevant for understanding the mode and site of action of polymethylene tetraamines if one considers that the furan ring is present in furtrethonium, which is a well-known muscarinic agonist. Examination of stereomodels of 1 and 18 as well as 19 revealed that the aromatic and heteroaromatic rings are superimposable and that the ring oxygen corresponds to the carbon at either position 2 or position 6 in the phenyl ring, owing to the relatively free rotation about the C–C bond between the ring and the benzylic carbon. The fact that 18 and 19 have a lateral chain α to the furan oxygen and that in polymethylene tetraamines bearing benzyl-type substituents optimum activity is associated with ortho substitution might indicate that a $\pi-\pi$ interaction between the aromatic moieties of 1 and 18 or 19 and a receptor electron-rich component requires increased electron density on the aromatic carbon bearing the tetraamine backbone. It is well-known that electron density in the furan system[34] is higher at C2 carbons whereas in substituted methoxybenzenes it is higher at positions 2 and 4, owing to the resonance hybrid of different canonical structures. The significant decrease in activity observed with 9 bearing a 4-methoxy function could be the result of an unfavorable steric effect. Furthermore, the low activity displayed by 20 and 21, bearing a pyridyl moiety, is in agreement with the above reasoning since the pyridine ring has a high electron density on the nuclear nitrogen and a low electron density at the other positions, mainly positions 2 and 4.

Another interesting finding was that relating to N-methylation of 1, giving the analogues 22–24. It turned out that N-methylation of the inner or outer nitrogens of 1 has a significantly different effect on activity. In fact, 23 proved to be one of the most active polymethylene tetraamines toward cardiac M-2 muscarinic receptors whereas analogue 22 was about 16-fold less active. This clearly indicates that the outer nitrogens of 1 play a crucial role in receptor binding and these nitrogens must be secondary amine function for optimum occupancy of cardiac M-2 muscarinic receptors whereas the inner nitrogens can be either secondary or tertiary moieties. In agreement with this view, tetramethylation of 1, giving 24, resulted in a significant decrease in activity, indicating that the beneficial effect on activity following N-methylation of the inner nitrogens is overwhelmed by the detrimental effect caused by N-methylation of the outer nitrogens.

Finally, it should be noted that all compounds proved to discriminate significantly between cardiac and ileal muscarinic receptors. These findings strengthen the notion that polymethylene tetraamines are a novel class of antagonists displaying unprecedented selectivity toward multiple subtypes of muscarinic receptors.[19-27]

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Although the IR and NMR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. The term "dried" refers to the use of anhydrous sodium sulfate.

***N*-[(*tert*-Butyloxy)carbonyl]-1,ω-alkanediamine.** *N*-[(*tert*-Butyloxy)carbonyl]-1,5-pentanediamine and *N*-[(*tert*-butyloxy)carbonyl]-1,7-heptanediamine were obtained as free bases in 15–20% yields from 2-[[[(*tert*-butyloxy)carbonyl]oxy]imino]-2-phenylacetonitrile (BOC-ON) and 1,5-pentanediamine or 1,7-heptanediamine following the procedure reported for *N*-[(*tert*-butyloxy)carbonyl]-1,6-hexanediamine.[29] They were used in the next step without further purification.

Synthesis of Diamine Diamide 25–29. The procedure adopted for the synthesis of 25 is described.

Ethyl chlorocarbonate (0.68 g, 6.28 mmol) was added dropwise to a stirred and cooled (5 °C) solution of 1,8-octanedioic acid (0.55 g, 3.14 mmol) and Et_3N (0.64 g, 6.28 mmol) in dioxane (100 mL), followed after 30 min by the addition of a solution of *N*-[(*tert*-butyloxy)carbonyl]-1,5-pentanediamine (1.27 g, 6.28 mmol) in dioxane (50 mL). After standing overnight, the mixture was poured into water (200 mL), and the white solid was filtered and washed with 2 N NaOH and water to give 1.07 g of residue, which was dissolved in EtOH (10 mL) and treated with HCl gas for 5 min. Removal of the solvent gave a residue that was dissolved in water and continuously extracted with chloroform for 8 h. The extracts were evaporated, yielding 0.54 g of 25 (mp 134–137 °C).

Similarly, compounds 26 (mp 146–149 °C), 27 (mp 133–135 °C), 28 (mp 126–128 °C), and 29 (mp 135–137 °C) were obtained in 45–55% yields from *N*-[(*tert*-butyloxy)carbonyl]-1,7-heptanediamine and 1,8-octanedioic acid, *N*-[(*tert*-butyloxy)carbonyl]-1,6-hexanediamine[29] and 1,11-undecanedioic acid, 1,13-tridecanedioic acid, or 1,14-tetradecanedioic acid, respectively. Compounds 25–29 were used in the next step without further purification.

Synthesis of Unsubstituted Tetraamine Tetrahydrochlorides 30–34. The procedure adopted for the synthesis of 30 is described.

Compound 25 (0.54 g, 1.58 mmol) was suspended in dry diglyme (100 mL) and treated with a 10 M solution of BH_3·MeSMe (0.5 mL) in dry diglyme (5 mL). After 14 h at 120 °C under a stream of dry nitrogen, excess borane was destroyed by careful addition of MeOH (3 mL). The resulting mixture was left to stand for 5 h, treated with HCl gas for 15 min, and then heated at 120 °C for 4 h to give a white solid that was filtered and crystallized from

(33) Van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299.
(34) Bak, B. *Acta Chem. Scand.* **1955**, *9*, 1355.

Table II

compd	mp,[a] °C	recrystn solvent	formula[b]
2	192–194	EtOH	$C_{39}H_{68}N_4O_2 \cdot 4HCl \cdot 2H_2O$
3	200–202	EtOH	$C_{41}H_{72}N_4O_2 \cdot 4HCl \cdot 0.5H_2O$
4	196–199	EtOH	$C_{42}H_{74}N_4O_2 \cdot 4HCl \cdot 0.5H_2O$
5	158–161	MeOH/Et_2O	$C_{34}H_{58}N_4O_2 \cdot 4HCl$
6	188–191	MeOH/2-PrOH	$C_{38}H_{66}N_4O_2 \cdot 4HCl \cdot 0.5H_2O$
7	>300	MeOH/H_2O	$C_{34}H_{58}N_4 \cdot 4HCl$
8	279–280	MeOH	$C_{36}H_{62}N_4O_2 \cdot 4HCl$
9	>300	MeOH/H_2O	$C_{36}H_{62}N_4O_2 \cdot 4HCl \cdot 0.5H_2O$
10	274–275	MeOH	$C_{36}H_{62}N_4 \cdot 4HCl \cdot 0.5H_2O$
11	>300	MeOH/H_2O	$C_{36}H_{62}N_4 \cdot 4HCl$
12	260–261	MeOH/2-PrOH	$C_{34}H_{56}Cl_2N_4 \cdot 4HCl \cdot 0.5H_2O$
13	>300	MeOH/H_2O	$C_{34}H_{56}Cl_2N_4 \cdot 4HCl$
14	>300	MeOH/H_2O	$C_{34}H_{56}Cl_2N_4 \cdot 4HCl \cdot 0.5H_2O$
15	225–227	MeOH	$C_{36}H_{62}N_4S_2 \cdot 4HCl \cdot 0.5H_2O$
16	262–263	MeOH	$C_{34}H_{56}N_6O_4 \cdot 4HCl$
17	>300	MeOH/H_2O	$C_{34}H_{56}F_2N_4 \cdot 4HCl \cdot 1.5H_2O$
18	>300	EtOH	$C_{30}H_{54}N_4O_2 \cdot 4HCl \cdot 1.5H_2O$
19	219–220	MeOH/H_2O	$C_{32}H_{58}N_4O_2 \cdot 4H_2C_2O_4$
20	261–262	MeOH/2-PrOH	$C_{32}H_{56}N_6 \cdot 4HCl \cdot H_2O$
21	294–295	MeOH/2-PrOH	$C_{32}H_{56}N_6 \cdot 4HCl \cdot 2.5H_2O$
22	119–121	MeOH/H_2O	$C_{38}H_{66}N_4O_2 \cdot 4H_2C_2O_4 \cdot 0.5H_2O$
23	167–169	MeOH	$C_{38}H_{66}N_4O_2 \cdot 4H_2C_2O_4 \cdot H_2O$
24	152–153	EtOH/MeOH	$C_{40}H_{70}N_4O_2 \cdot 4H_2C_2O_4 \cdot 2H_2O$

[a] The heating rate was 1 °C/min. [b] Analyses for C, H, N were within ±0.4% of the theoretical values required.

MeOH to give **30**: 0.34 g; mp >300 °C.

Similarly, **31–34** were obtained in 65–70% yield from **26–29**. Compounds **30–34** were used in the next step without further purification.

Synthesis of *N*-Aryl-Substituted Tetraamines 2-21. The procedure adopted for the synthesis of **2** is described.

A solution of **32** as the free base (0.31 g, 1.0 mmol) and 2-methoxybenzaldehyde (0.29 g, 2.1 mmol) in benzene (50 mL) was heated under reflux and the water formed continuously removed for 8 h. The cooled mixture was filtered and the filtrate evaporated to give the corresponding Schiff base that was dissolved in EtOH (50 mL) and treated with $NaBH_4$ (0.1 g). The mixture was stirred at room temperature for 3 h and then it was acidified with concentrated HCl (ice added) and evaporated to dryness. The residue was mixed with water (50 mL) and the mixture extracted with chloroform (2 × 30 mL) in order to remove nonbasic materials. The aqueous layer was made basic with NaOH pellets and the mixture extracted with chloroform (3 × 50 mL). The extracts were dried and then evaporated to give **2** as the free bases.

Similarly, **3–21** (as free bases) were obtained from the appropriate tetraamine **30**, **31**, **33**, **34** and *N,N'*-bis(6-aminohexyl)-1,8-octanediamine[19] (as free bases) and arylaldehyde. Compounds **2–18**, **20**, and **21** were characterized as the tetrahydrochloride salts and **19** as the tetraoxalate salt. The isolated yields ranged from 40 to 60% (Table II).

***N,N'*-Bis[6-[(2-methoxybenzyl)amino]caproyl]-1,8-octanediamine.** A solution of *N,N'*-bis(6-aminocaproyl)-1,8-octanediamine[19] (1.12 g, 3.02 mmol) and 2-methoxybenzaldehyde (0.91 g, 6.65 mmol) in MeOH (25 mL) was heated under reflux for 30 min, and then the solution was left at room temperature for 3 h with stirring. The cooled solution was treated with $NaBH_4$ (0.25 g) and left at room temperature for 6 h with stirring, and then it was acidified with 6 N HCl (10 mL). Removal of the solvent gave a residue that was taken up in water (200 mL) and the mixture extracted with ether (3 × 50 mL) to remove nonbasic materials. The aqueous layer was made basic with 2 N NaOH and the mixture extracted with chloroform (4 × 50 mL). The extracts were dried and then evaporated to give 1.89 g of the desired compound, which was used in the next step without further purification.

***N,N'*-Bis[6-[[(2-methoxybenzyl)methyl]amino]hexyl]-1,8-octanediamine Tetraoxalate (22).** A mixture of *N,N'*-bis[6-[(2-methoxybenzyl)amino]caproyl]-1,8-octanediamine (1.89 g), HCOOH (5 mL), and 36% HCHO (5 mL) was heated under reflux for 6 h. After cooling, the mixture was made basic with 20% NaOH and extracted with chloroform (3 × 50 mL). The extracts were dried and then evaporated to give 1.93 g of the corresponding dimethyl intermediate that was reduced with 10M BH_3·MeSMe (1.2 mL) as described for **30–34**, yielding crude **22**. It was purified as the free base by column chromatography by eluting with MeOH–28% ammonia (99:1) to give 0.15 g of pure compound that was characterized as the tetraoxalate salt (Table II).

***N,N'*-Bis[6-[[(benzyloxy)carbonyl]amino]caproyl]-*N,N'*-dimethyl-1,8-octanediamine.** This compound (mp 67–68 °C) was obtained in 70% yield from *N,N'*-dimethyl-1,8-octanediamine[30] and *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid via the procedure described for the *N*-butyloxycarbonyl derivative of **25**. It was used in the next step without further purification.

***N,N'*-Bis(6-aminocaproyl)-*N,N'*-dimethyl-1,8-octanediamine.** *N,N'*-Bis[6-[[(benzyloxy)carbonyl]amino]caproyl]-*N,N'*-dimethyl-1,8-octanediamine (4.0 g) in MeOH (180 mL) was hydrogenated over 10% Pd on charcoal (0.5 g) for 60 h at room temperature and a pressure of 30 psi. Following catalyst removal, the evaporation of the solvent gave 3.1 g of the desired compound, which was used in the next step without further purification.

***N,N'*-Bis(6-aminohexyl)-*N,N'*-dimethyl-1,8-octanediamine Tetrahydrochloride.** This compound was obtained in 50% yield by reduction of *N,N'*-bis(6-aminocaproyl)-*N,N'*-dimethyl-1,8-octanediamine with BH_3·MeSMe as described for **30–34**. It was purified by crystallization from EtOH-Et_2O: mp 169–171 °C.

***N,N'*-Bis[6-[(2-methoxybenzyl)amino]hexyl]-*N,N'*-dimethyl-1,8-octanediamine Tetraoxalate (23).** It was obtained in 65% yield from *N,N'*-bis(6-aminohexyl)-*N,N'*-dimethyl-1,8-octanediamine (as free base) and 2-methoxybenzaldehyde as described for **2** (Table II).

***N,N'*-Bis[6-[[(2-methoxybenzyl)methyl]amino]hexyl]-*N,N'*-dimethyl-1,8-octanediamine Tetraoxalate (24).** A mixture of **1**[19] (as free base) (0.29 g, 0.5 mmol), HCOOH (5 mL), and 36% HCHO (5 mL) was heated under reflux for 12 h. After cooling, the mixture was made basic with 20% NaOH and extracted with chloroform (3 × 50 mL). The extracts were dried and then evaporated to give 0.31 g of **24** as the free base that was transformed into the tetraoxalate salt and recrystallized (Table II).

Pharmacology. General Considerations. Guinea pigs (200–300 g) or rats (150–200 g) were sacrificed by cervical dislocation, and the organs required were rapidly set up under 1-g tension in 20-mL organ baths containing physiological salt solution (PSS) kept at the appropriate temperature (see below) and aerated with 5% CO_2–95% O_2. The composition of PSS was as follows (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.18; $CaCl_2$, 2.52; KH_2PO_4, 1.18; $NaHCO_3$, 23.8; glucose, 11.7. Dose–response curves were constructed by cumulative addition of the agonist. The concentration of agonist in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded

by means of a force transducer connected to a two-channel Gemini polygraph.

Guinea Pig Left Atria. The heart of male guinea pigs was rapidly removed, and the right and left atria were excized separately. Left atria were mounted in PSS at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 4-7 V). Inotropic activity was recorded isometrically.

Tissues were equilibrated for 1 h, and cumulative dose-response curves to carbachol (0.01-3 μM) were constructed. Following incubation with the antagonist for 30 min, a new dose-response curve to the agonist was obtained.

Guinea Pig Ileum. Twenty-millimeter-long portions of terminal ileum were taken at about 5 cm from the ileum-cecum junction and mounted in PSS at 37 °C. Tension changes were recorded isotonically.

Tissues were equilibrated for 30 min, and dose-response curves to carbachol (0.01-10 μM) were obtained at 30-min intervals, the first one being discarded and the second one taken as control. Following incubation with the antagonist for 30 min, a new dose-response curve to the agonist was obtained.

Determination of Dissociation Constants. The antagonist potency of compounds **1**-**24** at muscarinic receptors was expressed in terms of their pA_2 values. pA_2 values were estimated by Schild plots[31] constrained to slope -1.0, as required by theory,[32] by calculating the ratio of the doses (DR) of agonist causing 50% of the maximal response in the presence and in the absence of the test compound. The log (DR - 1) was calculated at three antagonist concentrations, and each concentration was tested at least five times. When this method was applied, it was always verified that the experimental data generated a line whose derived slope was not significantly different from unity ($p > 0.05$). For certain compounds pA_2 values were calculated at only one concentration, tested at least five times, according to van Rossum.[33]

Data are presented as means ± SE of n experiments. Differences between mean values were tested for significance by the Student's t test, and a level of $p > 0.05$ was taken as being statistically significant.

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Registry No. 1, 104807-40-1; **2**, 115962-36-2; **2** (unreduced), 115962-69-1; **2**·4HCl, 115962-78-2; **3**, 115962-37-3; **3**·4HCl, 115962-79-3; **4**, 115962-38-4; **4**·4HCl, 115962-80-6; **5**, 115962-39-5; **5**·4HCl, 115962-81-7; **6**, 115962-40-8; **6**·4HCl, 115962-82-8; **7**, 115962-41-9; **7**·4HCl, 115962-83-9; **8**, 115962-42-0; **8**·4HCl, 115962-84-0; **9**, 115962-43-1; **9**·4HCl, 115962-85-1; **10**, 115962-44-2; **10**·4HCl, 115962-86-2; **11**, 115962-45-3; **11**·4HCl, 115962-87-3; **12**, 115962-46-4; **12**·4HCl, 115962-88-4; **13**, 115962-47-5; **13**·4HCl, 115981-87-8; **14**, 115962-48-6; **14**·4HCl, 115962-89-5; **15**, 115962-49-7; **15**·4HCl, 115962-90-8; **16**, 115962-50-0; **16**·4HCl, 115962-91-9; **17**, 115962-51-1; **17**·4HCl, 115962-92-0; **18**, 115962-52-2; **18**·4HCl, 115981-85-6; **19**, 115962-53-3; **19**·4HCl, 115981-86-7; **20**, 115962-54-4; **20**·4HCl, 115962-93-1; **21**, 115962-55-5; **21**·4HCl, 115962-94-2; **22**, 115962-56-6; **22**·$4H_2C_2O_4$, 115962-95-3; **23**, 115962-57-7; **23**·$4H_2C_2O_4$, 115962-76-0; **24**, 115962-58-8; **24**·$4H_2C_2O_4$, 115962-77-1; **25**, 115962-59-9; **25** (BOC blocked), 115962-68-0; **26**, 115962-60-2; **27**, 115962-61-3; **28**, 115962-62-4; **29**, 115981-14-1; **30**, 115962-63-5; **31**, 115962-64-6; **32**, 115962-65-7; **33**, 115962-66-8; **34**, 115962-67-9; 1,8-octanedioic acid, 505-48-6; *N*-[(*tert*-butyloxy)carbonyl]-1,5-pentanediamine, 51644-96-3; *N*-[(*tert*-butyloxy)carbonyl]-1,7-heptanediamine, 99733-18-3; *N*-[(*tert*-butyloxy)carbonyl]-1,6-hexanediamine, 51857-17-1; 1,11-undecanedioic acid, 1852-04-6; 1,13-tridecanedioic acid, 505-52-2; 1,14-tetradecanedioic acid, 821-38-5; 2-methoxybenzaldehyde, 135-02-4; 3-methoxybenzaldehyde, 591-31-1; 4-methoxybenzaldehyde, 123-11-5; 2-methylbenzaldehyde, 529-20-4; 4-methylbenzaldehyde, 104-87-0; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 4-chlorobenzaldehyde, 104-88-1; 2-(methylthio)benzaldehyde, 7022-45-9; 4-nitrobenzaldehyde, 555-16-8; 4-fluorobenzaldehyde, 459-57-4; 2-furancarboxaldehyde, 98-01-1; 5-methyl-2-furancarboxaldehyde, 620-02-0; 2-pyridinecarboxaldehyde, 1121-60-4; 3-pyridinecarboxaldehyde, 500-22-1; benzaldehyde, 100-52-7; *N*,*N*′-bis(6-aminohexanyl)-1,8-octanediamine, 104807-24-1; *N*,*N*′-bis[6-[(2-methoxybenzyl)amino]caproyl]-1,8-octanediamine, 115962-71-5; *N*,*N*′-bis(6-aminocaproyl)-1,8-octanediamine, 115962-70-4; *N*,*N*′-bis[6-[(2-methoxybenzyl)methylamino]-caproyl]-1,8-octanediamine, 115962-72-6; *N*,*N*′-bis[6-[(2-methoxybenzyl)amino]caproyl]-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-73-7; *N*,*N*′-dimethyl-1,8-octanediamine, 33563-54-1; *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid, 1947-00-8; *N*,*N*′-bis(6-aminocaproyl)-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-74-8; *N*,*N*′-bis(6-aminohexyl)-*N*,*N*′-dimethyl-1,8-octanediamine tetrahydrochloride, 115981-84-5; *N*,*N*′-bis(6-aminohexyl)-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-75-9.

Inhibition of ^{125}I-Labeled Ristocetin Binding to *Micrococcus luteus* Cells by the Peptides Related to Bacterial Cell Wall Mucopeptide Precursors: Quantitative Structure-Activity Relationships

Ki-Hwan Kim,* Yvonne Martin, Ellen Otis, and James Mao

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064. *Received December 11, 1987*

Quantitative structure-activity relationships (QSAR) of *N*-Ac amino acids, *N*-Ac dipeptides, and *N*-Ac tripeptides in inhibition of ^{125}I-labeled ristocetin binding to *Micrococcus luteus* cell wall have been developed to probe the details of the binding between ristocetin and N-acetylated peptides. The correlation equations indicate that (1) the binding is stronger for peptides in which the side chain of the C-terminal amino acid has a large molar refractivity (MR) value, (2) the binding is weaker for peptides with polar than for those with nonpolar C-terminal side chains, (3) the N-terminal amino acid in *N*-Ac dipeptides contributes 12 times that of the C-terminal amino acid to binding affinity, and (4) the interactions between ristocetin and the N-terminal amino acid of *N*-acetyl tripeptides appear to be much weaker than those with the first two amino acids.

For the past 25 years medicinal chemists have sought statistical relationships between the physical and biological properties of molecules. Until recently such quantitative structure-activity relationships (QSAR) were developed without knowledge of the structure of the target of the drug. Hence, most QSAR cannot be checked against a structure of the drug-receptor complex.

We have been involved in an investigation of the complex between the antibiotic ristocetin (Figure 1) and Ac_2-L-Lys-D-Ala-D-Ala, a model of the bacterial cell wall as reported here.

Ristocetin is a glycopeptide antibiotic of the vancomycin group[1] isolated from the microorganism *Nocardia lurida*.[2] It is a linear heptapeptide, cross-linked between residues 1 and 2, 2 and 4, and 4 and 6 by diphenyl ether bridges

(1) (a) Wallas, C. H.; Strominger, J. L. *J. Biol. Chem.* **1953**, 238. (b) Williams, D. H. *Acc. Chem. Res.* **1984**, *17*, 364.

(2) Grundy, W. E.; Sinclair, A. C.; Theriault, R. J.; Goldstein, A. W.; Rickher, C. J.; Warren, H. B., Jr.; Oliver, T. J.; Sylvester, J. C. *Antibiotics Annual 1956-1957*, Welch, H., Marti-Ibanez, F., Eds.; Medical Encyclopaedia Inc.: New York; p 687.

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