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Studies on the Cholinergic Receptor. 6.[1] Synthesis and Muscarinic Activity of 2-Methyl-4-(2-dimethylaminoethyl)-1,3-dioxolane Methiodide[2]

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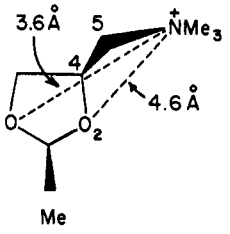
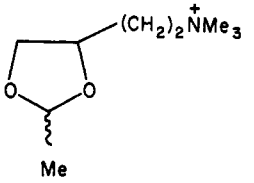
Previous studies[3a-c] utilizing conformationally restricted 1,3-dioxolane analogs of the highly potent muscarinic agent I have suggested that the "active" conformation of I is that in which the N^+Me_3 group is maximally extended from O_1 and O_3. Some further confirmation of this is offered by the finding that II (approximately 80% cis, 20% trans) in which the N^+Me_3 group can sweep an area significantly greater than in I but cannot attain conformation I is very significantly less active than I (ED_{50}, I, 3×10^{-8} *M*; II, 1.9×10^{-5} *M*; *inter alia*, I and II = 1).



I



II

It is of interest that the conformation I deduced by us on the basis of conformationally restricted analogs is in reasonable agreement with that obtained for *cis*-2(*S*)-methyl-4(*R*)-dimethylaminomethyl-1,3-dioxolane methiodide by Pauling and Petcher through X-ray analysis[4] (torsion angle, $O_2C_4C_5N^+$, +94°, $N^+ \rightarrow O_1$, 3.2 Å, $N^+ \rightarrow O_2$ 4.79 Å). However, a number of arguments can be advanced[1,5,6] to suggest quite strongly that there is not a single unique binding conformation for muscarinic agonists: hence, the conformation shown in I may be quite irrelevant to the binding conformations of other agents, particualrly if they are structurally unrelated.

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Experimental Section

Chemistry.—Melting points were determined on a Thomas-Kofler hot stage and are corrected. Nmr spectra were recorded with a Varian A-60; glpc analyses were carried out with a 10% Carbowax column using an F and M Research Chromatograph (Model 5750). Elemental analyses were by Dr. A. E. Bernhardt and, where indicated only by symbols of the elements, are within ±0.4% of the theoretical values.

2,2-Dimethyl-4-(2-hydroxyethyl)-1,3-dioxolane was prepd in 46% yield from acetone (6.4 g, 0.11 mole), 1,2,4-trihydroxybutane (10.6 g, 0.1 mole), and *p*-TsOH (0.05 g) in refluxing PhH (50 ml) with azeotropic removal of H_2O and had bp 52–55° (0.2 mm); nmr (neat, Me_4Si), 2-CH_3, τ 8.66, 8.74 (singlets, cis and trans, respectively, to the 4 substituent), CH_2CH_2OH, 8.21 (asymmetric quartet), multiplets at 6.36, and 5.91. *Anal.* ($C_7H_{14}O_3$) C, H.

2-Methyl-4-(2-dimethylaminoethyl)-1,3-dioxolane Methiodide (II).—2,2-Dimethyl-4-(2-hydroxyethyl)-1,3-dioxolane (0.1 mole) was converted to the chloro compound by treatment in $CHCl_3$ (50 ml) with an equimolar amt of $SOCl_2$ at 0°. The mixt was stirred at 35° for 120 min, and then refluxed with an equal vol of MeOH for 15 min and stripped *in vacuo*. The residue was taken up in $CHCl_3$, washed (aq K_2CO_3), dried, and stripped to give crude 4-chloro-1,2-dihydroxybutane which was converted to 2-methyl-4-(2-chloroethyl)-1,3-dioxolane by reaction with paraldehyde in refluxing PhH with azeotropic removal of H_2O; this had bp 56° (15 mm); nmr (neat, Me_4Si), 2-CH_3, τ 8.71 (major doublet, cis), 8.75 (minor doublet, trans), 2-H, 5.0 (unsymmetrical quartet). *Anal.* ($C_6H_{11}ClO_2$) C, H, Cl. 2-Methyl-4-(2-chloroethyl)-1,3-dioxolane was treated with Me_2NH in PhH at 100° for 24 hr and subsequently quaternized with MeI in Et_2O to give II (65%) as colorless prisms with mp 148–151°; nmr (CD_3CN, Me_4Si), 2-CH_3, τ, 8.65 (major doublet, cis), 8.70 (minor doublet, trans). 2-H, 5.0 (overlapping quartets), $N^+(CH_3)_3$, 6.80. *Anal.* ($C_9H_{20}INO_2$), C, H, I, N.

Biology.—Muscarinic activities were determined using the rat jejunum as previously described.[3a-c]

Potential Folic Acid Antagonists. 5. Synthesis and Dihydrofolate Reductase Inhibitory Activities of 2-Amino-4,6-substituted-5-arylazopyrimidines

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Our previous studies of the structural requirements of 5-arylazopyrimidines[1] for inhibitory activity toward dihydrofolate reductase have been largely concerned with 2,4,6-triamino-5-arylazopyrimidines. Optimum activity was found with 2,4,6-triamino-5-(2 ethylphenyl)azopyrimidine.[2] We now report the effect of additional substitution in the pyrimidine ring.

The data in Table I show, in accord with much previous work,[3,4] that significant activity is associated with the 2,4-diaminopyrimidine nucleus. However, optimum activity is found with the 2,4-diamino-6-hydroxypyrimidine nucleus (**4** and **5**) an observation contrasting

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TABLE I

2-AMINO-4,6-SUBSTITUTED-5-ARYLAZOPYRIMIDINES

No.	Substituent 4	Substituent 6	Substituent 5	Mp, °C	Formula	Analysis	$([I]/[S]_{0.5})$
1	NH_2	NH_2	C_6H_5	261[a]		[c]	0.31
2	NH_2	H	C_6H_5	275[a]	$C_{10}H_{10}N_6$	C, H, N	1.00
3	NH_2	CH_3	C_6H_5	216[a,b]	$C_{11}H_{12}N_6$	C, H, N[d]	0.20
4	NH_2	OH	C_6H_5	>320[c]	$C_{10}H_{10}N_6O$	*c*, *e*	0.063
5	NH_2	OH	2-$C_2H_5C_6H_5$	>360[b]	$C_{12}H_{14}N_6O$	C, H, N	0.010
6	OH	OH	C_6H_5	>360[b]	$C_{10}H_9N_5O_2$	C, H, N	>4.0
7	OH	OH	2-$C_2H_5C_6H_5$	>360[b]	$C_{12}H_{13}N_5O_2$	C, H, N	>4.0

[a] Recrystd from *i*-PrOH. [b] Recrystd from DMF. [c] Standard compd, ref 1. [d] Lit. [K. Tanaka, E. Omura, T. Sugawa, Y. Sanno, Y. Ando, K. Imai, and M. Kawashima, *Chem. Pharm. Bull.*, **7**, 1 (1959)] mp 224–226°. [e] Lit. mp >300° (F. R. Benson, L. W. Hartzel, and W. L. Savell, *J. Amer. Chem. Soc.*, **72**, 1816 (1950).

with those of Baker, *et al.*,[5] comparing 2,4,6-triamino- and 2,4-diamino-6-hydroxy-5-alkylpyrimidines on pigeon liver dihydrofolate reductase. Elimination of the 6-amino function (**2**) reduces its activity while its replacement by Me (**3**) increases activity but not to the same extent as does the 6-OH group (**4**). Of particular interest is the finding that introduction of the *o*-Et group into 2,4-diamino-6-hydroxy-5-phenylazopyrimidine produces a 6-fold increase in activity, thus paralleling the effects of the same substitution into 2,4,6-triamino-5-phenylazopyrimidine. The similarity of these substituent effects suggests quite strongly that the binding orientations of the 2,4,6-triamino- and 2,4-diamino-6 hydroxy-5-phenylazopyrimidines on dihydrofolate reductase are identical or very closely similar.

Experimental Section[6]

Synthetic Procedure.—The compounds listed in Table I were prepd by coupling diazotized $PhNH_2$ and *o*-ethylaniline with the appropriate pyrimidine according to the methods previously described.[1,2]

Enzyme Procedure.—The inhibitory activities of the compds were detd with dihydrofolate reductase from chicken liver[7] using the procedure previously described.[2]

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Antigenic Polypeptides. Synthesis and Immunochemical Studies of Poly(L-phenylalanyl-L-glutamyl-L-alanylglycyl)-glycine-*1*-^{14}C Ethyl Ester

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A recent investigation of the immunochemical properties of poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester,[1,2] has shown that the polypeptide is antigenic, eliciting antibodies in rabbits.[3] A desire to ascertain the role of the phenolic OH group of the tyrosyl residue of this antigen on its immunochemical properties prompted the synthesis of poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**).

Chemistry. The polymerizing unit Phe-γ-*tert*-Bu-Glu-Ala-Gly pentachlorophenyl ester·HCl (**4**) and the necessary intermediates for its preparation were synthesized as detailed in the Experimental Section. The polymerization was performed at a reagent concn of 100 mmoles/l. in the presence of a preformed monomer since this has been shown to produce linear high molecular weight polypeptides.[1,2,4-8] Following this established procedure the insoluble polymer, poly(Phe-γ-*tert*-Bu-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester was prepared; from which the protecting *tert*-Bu groups were removed by the use of 90% F_3CCO_2H to yield poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**). After extensive dialysis, the polymer was purified and fractionated by passage through a calibrated column of Sephadex G-50.[9] By this means the mol wt of the polypeptide was found to be 1×10^4.

Immunochemistry.—Two rabbits were immunized with **1** using the same protocol as that previously described.[3] To aliquots of the sera obtained from each rabbit were added incremental amounts of the synthetic polypeptide **1**. A precipitin reaction was observed for one of the rabbits, as shown in Figure 1.

Conclusions.—It has been reported that tyrosine and phenylalanine are equally effective in enhancing antibody formation in random copolymers.[10] Previously, we have cast some doubt on this finding being applicable for linear sequential polypeptides.[11] However, from subsequent work it has now been found that replacement of the tyrosyl residue with the phenylalanyl moiety in the antigen, poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester, still affords an antigenic polypeptide. Thus it has been concluded that the phenolic OH group is not a necessity in order to confer antigenicity to a molecule.

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