pH 8.0 and 37°. The frozen aqueous soln contains approx 5 mg of $(NH_4)_2SO_4$/mg of protein.)

ACh Chloride Soln. Because of the extremely hygroscopic nature of AChCl, stock solutions were prepared as follows. About 1 g of AChCl (cryst, "99%," Sigma Chemical Co) was transferred under N_2 in a glove box to each of several weighing bottles. These bottles were stoppered, weighed, and stored in a freezer until needed. Just prior to a kinetic run a 2.015 *M* stock soln is obtained by adding to one of the bottles an amount of H_2O weighing 1.8013 times the number of g of AChCl. The density of such a soln was previously determined to be 1.0268 g/ml at 20°; 1.026 g (1 ml) of stock is removed and diluted to 10 ml giving a 0.2015 *M* soln; 8 ml of this soln is removed and diluted to 10 ml giving a 0.1613 *M* soln. Repeating this dilution technique 4 times gives 4 more solutions: 0.1291 *M*, 0.1033 *M*, 0.0826 *M*, and 0.0661 *M*.

Isomeric *dl*-3-Trimethylammonium-2-acetoxy-*trans*-decalin Halides. The preparation and properties of these compds have been described previously.[1]

Isomeric-*dl*-1-Methyl-3-acetoxy-*trans*-decahydroquinoline Methiodides. A description of these compds has likewise appeared previously.[2]

Analog Substrate Soln. A stock soln (2 ml) (approx 0.2 *M*) of each of the 6 compds was prepd. Five successive dilutions were made by removing 0.8 ml of the previous soln and diluting to 1 ml with distilled H_2O. Six solns for kinetic study are thus obtained.

Enzyme Kinetics. The rate of AChE hydrolysis of AChCl and its analogs was followed by measuring the rate of AcOH production by the pH Stat method. The Radiometer Co. TTT 11 Titrator, ABU1 Auto-burette fitted with a 0.25-ml buret, SBR2C Titrograph recorder, PHM26 expanded scale pH meter with Type 202C glass electrode and Type K 401 calomel electrodes, and a TTA3 Titration Assembly equipped with a constant temperature anaerobic assay chamber and motor driven stirrer were used.

Measurements were made under N_2 at pH 7.2 ± 0.1 at 24.90 ± 0.05° using enzyme strengths of 0.1 to 10 μM units/ml and 6 different concns (ranging from $3 \times 10^{-4} M$ to $10 \times 10^{-4} M$) of substrate. At least 3 runs were performed for each of the 6 concns of each of the 7 substrates (AChCl and 6 analogs). The titrant was 0.0100 *N* NaOH. In a typical run 10 ml of freshly prepared enzyme soln (kept under N_2) is removed to the thermostated assay chamber with a volumetric pipet and allowed to reach constant temp. To this soln, under N_2, is added 0.050 ml of substrate soln, and the instrument is activated. The end-point pH is set at 7.3 with a proportional band setting of 0.2.

The raw data consisting of a chart trace of per cent full buret *vs.* centimeters are punched onto cards. Reaction velocities are extrapolated to 0 time to give the initial reaction velocity for each substrate concn. An iterative least-squares fit directly to the Michaelis equation affords values for K_m and V_{max}.

Base-Hydrolysis Kinetics. Rates were studied at 24.90 ± 0.05° using 10.00 ml of reaction soln containing substrate concn of approximately $6 \times 10^{-4} M$. At least 4 runs were made for each substrate at each of 4 different base concns ranging from pH 10.0 to 11.0. The base concn was kept constant (±0.01 pH unit) by the addition of 0.0500 *N* NaOH as described in the previous section. Electrodes were calibrated for the pH range 9–12 at various temp with commercial buffer soln and standard NaOH soln; corrections at 25° were found not to be required. Blank runs (no base present) gave negligible rates. Using the relationship between rate constant and ionic strength derived by Robson-Wright for ACh hydrolysis, it was calculated that any variations in ionic strength occurring under our conditions either (a) during a run or (b) between runs at different base concns have a negligible effect (within experimental error) on our rate constant values.

A typical determination is carried out as follows. A NaOH soln (10.00 ml) of approx the desired pH (about 0.1 pH unit higher than the preset end point) is added to a thermostated reaction vessel and stirred under N_2 until const temperature is reached. To this soln is added 0.100 ml of a 0.06 *M* substrate stock soln. The reaction is followed until 90% completion. Raw data (in the form of a recorder chart trace of per cent of full buret *vs.* centimeters) are converted to time and concn of titrant added (*i.e.*, of ester reacted) and fitted by a least-squares technique to the first-order rate equation. The resulting observed (pseudo-first-order) rate constants ($k_{obsd} = k_{OH}[OH^-]$) are plotted against base concn to give k_{OH} values.

Acknowledgment. The authors gratefully acknowledge support of this project by the National Institutes of Health Grants GM-9254 and CA 33233.

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Structural Parameters Determining Cholinergic and Anticholinergic Activities in a Series of 1,3-Dioxolanes

K. J. Chang, R. C. Deth, and D. J. Triggle*

Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214.
Received July 21, 1971

A series of 1,3-dioxolane-4-dimethylaminomethyl methiodides substituted at the 2 position with alkyl and aryl groups have been examined for their agonist and antagonist activities at muscarinic receptors in guinea pig ileal muscle and rat jejunum. Complete lack of stereoselectivity of antagonist activity was found with the enantiomers of the 2,2-diphenyl and the 2-phenyl-2-cyclohexyl derivatives. A reasonable correlation of pA_2 and π was observed. pA_2 values appeared independent of the agonist employed. Selected compounds were examined for their antihistaminic activities: these were 100–500 times less than the anticholinergic activities but were similarly nonstereoselective.

The 1,3-dioxolane nucleus serves as the basic structure for a number of potent agonists and antagonists active at the muscarinic acetylcholine receptors of smooth muscle.[1] 2-Methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (Table IV, 23) originally described by Fourneau, *et al.*,[2] is a particularly selective and active agonist at muscarinic receptors:[1,3,4] its interaction with the muscarinic receptor is highly stereospecific, the cis isomer being 5–10 times more potent than the trans isomer[3] and the 2*S*,4*R* isomer being 100 times more active than the 2*S*,4*S* isomer.[4,5]

In common with other agonist structures[1,6] 23 may be converted to parasympatholytic agents by the substitution of nonpolar groups. Thus, 2,2-diphenyl-4-dimethylaminomethyl-1,3-dioxolane methiodide has long been known as a potent parasympatholytic agent.[7]

Our previous studies with 1,3-dioxolanes, largely concerned with their utility as "rigid agonist analogs"[8a-e] had revealed,[8c] in contrast to the agonist activity of 23 that the cis and trans isomers of 2-phenyl-4-dimethylaminomethyl-1,3-dioxolane methiodide were not significantly different in antagonistic activity. This situation is similar to that described for a series of β-methylcholine derivatives[9a,b] where, in the agonist-antagonist transition, stereoselectivity of the choline portion of the molecule was lost. Such observations raise the distinct possibility that the binding sites of agonists and antagonists may be totally or partially distinct.[1,6,8c,9a,b] It thus appeared of interest to examine in some detail the structural and steric parameters of agonist and antagonist activity in a series of 1,3-dioxolanes. A principal objective was to determine, through the use of linear free energy relationships and stereoselectivity studies, whether the binding of the 2 substituents in agonists and antagonists based on the 1,3-dioxolane nucleus is at a common area, or whether discrete binding sites may exist for these substituents.[8c] After this work was completed Brimblecombe and Inch[10] reported their studies of a series of 1,3-dioxolanes including some of the compounds that we have studied. Where the same compounds have been studied our results show good agreement with those first reported by Brimblecombe and Inch.

Experimental Section†

Preparations of Compounds. The 2,2-dialkyl-4-chloromethyl-1,3-dioxolanes listed in Table I were prepared by reaction of the appropriate ketone and 1-chloropropane-2,3-diol in refluxing PhH with *p*-TsOH and azeotropic removal of H_2O.[8b] The 2,2-dialkyl-4-chloromethyl-1,3-dioxolanes were treated with Me_2NH–C_6H_6 at 100° for 6 hr in a sealed tube, excess Me_2NH was removed with a current of warm N_2, and the residual unpurified tertiary amines were quaternized with excess MeI–Et_2O[8b] to give 2,2-dialkyl-4-dimethylaminomethyl-1,3-dioxolane methiodides (Table I).

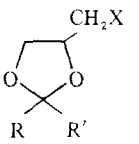
The 2,2-diaryl-4-dimethylaminomethyl-1,3-dioxolanes were prepared similarly from the 2,2-diaryl-4-tosyloxymethyl-1,3-dioxolanes: the latter were prepared from the corresponding ketone and 1-tosyloxyglycerol[4] in refluxing PhH with azeotropic removal of H_2O. The dioxolanes thus derived from 2-, 3-, and 4-methyl- and 4-hydroxybenzophenones were noncrystalline oils and were not characterized further, although ir spectra were consistent with their structure. Optically active compounds were prepared from D- and L-tosyloxyglycerol.[4,10] D-2,2-Diphenyl-4-tosyloxymethyl-1,3-dioxolane had mp 82–83° (PhH–ligroin), [α]D +25.1 (*c* 5, CCl_4). *Anal.* ($C_{23}H_{22}O_5S$) C, H. L-2,2-Diphenyl-4-tosyloxymethyl-1,3-dioxolane had mp 80–83°, [α]D −23.2 (*c* 5, CCl_4). *Anal.* ($C_{23}H_{22}O_{5S}$) C, H. The D- and L-2,2-diphenyl-4-dimethylaminomethyl-1,3-dioxolane methiodides had rotations ([α]D) of +11.7 and −10.8, respectively (*c* 5, MeOH).

Pharmacological Activities. Activities were determined using longitudinal muscle from the guinea pig ileum isolated according to Rang[11] and in a few experiments using rat jejunum.[8a-e] Muscle segments approximately 2 cm in length were suspended in jacketed muscle baths in 10 ml of Tyrode's solution (NaCl, 8 g/l; $CaCl_2 \cdot 2H_2O$, 0.26 g/l; $MgCl_2 \cdot 6H_2O$, 0.2 g/l; KCl, 0.2 g/l; $NaHCO_3$, 1.0 g/l; glucose, 1.0 g/l) aerated with air and held at 37–37.5° and 30–31° for longitudinal ileal muscle and rat jejunum, respectively. Isotonic contractions were recorded on smoked paper drums. The tensions on the longitudinal ileal muscle and jejunum were 0.35 g and 1.0 g, respectively.

pA_2 values were measured according to Schild's method.[12a,b] All of the antagonists studied showed purely reversible competitive behavior giving parallel shifts of the agonist concentration–response curves without depression of the maximum response. Washing of the antagonist from the tissue gave an immediate (1–2 min) restoration of original agonist sensitivity. After responses had become stable, cumulative dose–response curves of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide were obtained in the absence and presence of the antagonist: the incubation time for the antagonist was 5 min: in some experiments pentyltrimethylammonium iodide was used. Agonist and antagonist solutions were prepared as 10^{-2} *M* stock solutions in distilled H_2O and dild with Tyrode's soln for use. Three or 4 different concentrations of antagonist were studied in the same preparation. The regression line of log [DR − 1] *vs.* −log [I] was computed and pA_2 is the value of the intercept at the −log [I] ordinate. pA_2 values for some of the antagonists against histamine in the guinea pig longitudinal muscle were similarly determined. The pD_2 values of agonists and partial agonists are the negative logarithms of the ED_{50} values and were unchanged in the presence of hexamethonium (100 mg/1000 ml) indicating the absence of nicotinic effects. π (hydrophobicity) values for the compounds were calculated from the substituent constant compilation of Fujita, *et al.*[13] using an arbitrary value of π = 0 for the parent 4-dimethylaminomethyl-1,3-dioxolane nucleus common to all of the compounds studied. The pA_2, pD_2, and values so obtained are listed in Tables II, III, and IV.

†Melting points were recorded on a Thomas-Kofler hot stage and are corrected. Analyses were performed by Dr. A. E. Bernhardt and, where indicated only by symbols of the elements, were within 0.4% of the theoretical values.

Table I. Structure and Physical Properties of New 2,2-Disubstituted-1,3-dioxolanes



R	R'	X	Mp or bp (mm), °C	Anal.
R = R'				
$CH(CH_3)_2$		Cl	100–102 (17)	C, H, Cl
Cyclopropyl		Cl	80–81 (2.5)	C, H, Cl
$CH(CH_3)_2$		$N^+Me_3 \cdot I^-$	198–200	C, H, I, N
Cyclopropyl		$N^+Me_3 \cdot I^-$	138–140	C, H, I, N
C_6H_{11}		$N^+Me_3 \cdot I^-$	128–130	C, H, I, N
C_6H_5(R)		$N^+Me_3 \cdot I^-$	198–199	C, H, I, N
C_6H_5(S)		$N^+Me_3 \cdot I^-$	197–199	C, H, I, N
R ≠ R'				
C_6H_5	2-MeC_6H_4[a]	$N^+Me_3 \cdot I^-$	188–190	C, H, I, N
C_6H_5	3-MeC_6H_4[a]	$N^+Me_3 \cdot I^-$	165–175	C, H, I, N
C_6H_5	4-MeC_6H_4[a]	$N^+Me_3 \cdot I^-$	179–184	C, H, I, N
C_6H_5	4-HOC_6H_4[a]	$N^+Me_3 \cdot I^-$	110–125	C, H, I, N

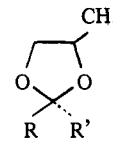
[a] Of undetermined cis, trans geometry.

Results and Discussion

The results listed in Table II confirm and extend previous observations[8c,10] of the lack of stereoselectivity of the 2-substituted-4-dimethylaminomethyl-1,3-dioxolane methiodides as muscarinic antagonists. This is seen very clearly with 1 and **2**, **4–6**, and **10–15**. The marked contrast to the interaction of the highly potent agonist 2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide was noted previously. This may indicate partial or total distinction of binding sites for agonists and antagonists; this point is treated in detail in existing discussions.[1,6,9a,b,14,15]

According to Schild[12a,b] agonists which act on the same receptors will produce the same pA_x value with competitive antagonists. However, if agonists and antagonists bind at different sites[1,6,8c,9a,b] and if, as suggested elsewhere,[1,15] there may exist different or partially different binding sites or binding orientations for agonists of different structural type then the observed antagonist activity (pA_2) *may* vary with the agonist structure since the allosteric linkage may not be equally transmitted to the various agonist sites. The cis dioxolane **23** and pentyltrimethylammonium were selected as agonists of differing type, but the data of Table

Table II. pA_2 Values for Antagonism by 1,3-Dioxolanes of Muscarinic and Histaminic Receptors



No.	R	R'	pA_2 ± SE Muscarinic[a] Rat Jej	pA_2 ± SE Muscarinic[a] Guinea pig Long.	pA_2 ± SE Histaminic[b] Guinea pig Long.	π
1	C_6H_5	H		4.61 ±0.04 (4)		1.89
2	H	C_6H_5		4.51 ± 0.01 (4)		1.89
3	C_6H_5	4-HOC_6H_4[c]		5.52 ± 0.02 (4)		3.17
4	C_6H_5	C_6H_5(RS)		7.66 ± 0.04 (6)	5.53 ± 0.04 (4)	3.78
5	C_6H_5	C_6H_5[d]	7.73 ± 0.02 (8)	7.55 ± 0.03 (6)	5.68 ± 0.06 (4)	3.78
6	C_6H_5	C_6H_5[e]	7.75 ± 0.04 (5)	7.62 ± 0.03 (6)	5.56 ± 0.03 (4)	3.78
7	C_6H_5	2-MeC_6H_4[c]	7.81 ± 0.10 (4)	7.74 ± 0.02 (4)		4.44
8	C_6H_5	3-MeC_6H_4[c]		7.28 ± 0.10 (4)		4.29
9	C_6H_5	4-MeC_6H_4[c]	7.21 ± 0.05 (6)	7.39 ± 0.02 (4)		4.30
10	C_6H_{11}	C_6H_5(*RS*)	8.00 ± 0.06 (5)	7.88 ± 0.03 (4)	5.48 ± 0.05 (4)	4.40
11	C_6H_5	C_6H_{11}(*RS*)	8.11 ± 0.04 (6)	8.12 ± 0.03 (7)	5.57 ± 0.07 (4)	4.40
12	C_6H_{11}	C_6H_5(2*S*, 4*S*)[e]		8.03 ± 0.03 (7)	5.39 ± 0.01 (4)	4.40
13	C_6H_{11}	C_6H_5(2*R*, 4*R*)[d]		7.73 ± 0.02 (3)		4.40
14	C_6H_5	C_6H_{11}(2*R*, 4*S*)[e]		7.83 ± 0.01 (4)		4.40
15	C_6H_5	C_6H_{11}(2*S*, 4*R*)[d]		8.26 ± 0.03 (4)		4.40
16	C_6H_{11}	C_6H_{11}		6.44 ± 0.04 (6)		5.02
17	$CH_3(CH_2)_3$	$CH_3(CH)_3$		6.59 ± 0.03 (4)		3.52
18	$CH_3(CH_2)_2$	$CH_3(CH)_2$		6.00 ± 0.04 (3)		2.86

[a] *cis*-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide was used as agonist. [b] Histamine was used as agonist. [c] Of undetermined cis, trans geometry. [d] Derived from L-glycerol-1-tosylate. [e] Derived from D-glycerol-1-tosylate.

Table III. pA_2 Values for Antagonist Activity Against *cis*-2-Methyl-4-dimethylaminomethyl-1,3-dioxolane Methiodide and *n*-Pentyltrimethylammonium

Antagonist	pA_2 ± SE Agonist[a] Dioxolane	$C_5H_{11}N^+Me_3$	Difference
12	8.03 ± 0.03 (7)	8.26 ± 0.07 (4)	$0.05 > P > 0.01$
14	7.83 ± 0.02 (4)	7.87 ± 0.05 (4)	$P > 0.05$
13	7.73 ± 0.02 (3)	7.82 ± 0.06 (3)	$P > 0.05$
6	7.62 ± 0.03 (6)	7.67 ± 0.05 (4)	$P > 0.05$

[a] Values in parentheses are number of observations.

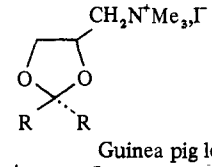
III, with the possible exception of the first entry do not indicate significant differences in pA_2 values. Brimblecombe, *et al.*,[16] have reported similarly using atropine and 5 agonists. It is possible, however, that if the major effect of an allosteric antagonist is directed to the ammonium binding site[15] of the agonist receptor then differences in antagonist activity will not be observed using agonists with a common ammonium function.

The compounds listed in Table II that are common to those first reported by Brimblecombe and Inch[10] (**1**, **2**, and **10–15**) show good agreement in biological activities. Thus, the order of activity found here and previously[10] is **15** > **12** > **14** > **13** and the racemates (**10** and **11**) have activities intermediate between their corresponding optical isomers.

The effects of substitution in the Ph ring of the 2,2-diphenyl-1,3-dioxolane series are of interest. Substitution of an OH group (**3**) reduces activity by ~100-fold, possibly by interfering with binding to a nonpolar area: substitution of the 2-, 3-, or 4-Me groups (**7–9**) results in maintained activity with the 2-Me group and reduced activity with the 3- and 4-Me groups. A rather similar effect of the same substitutions has been observed[17] in the diphenhydramine ($Ph_2CHOCH_2CH_2NMe_2$) series where the effects of ortho-alkyl substitution on anticholinergic activity are satisfactorily correlatable with E_S.[1]

Where comparisons are possible betwen the rat jejunum and the guinea pig ileal longitudinal muscle it is clear that the antagonist interactions are not significantly different. The antihistaminic activity of the 1,3-dioxolanes (Table II) has been noted previously[18] and is not surprising in view of the structural similarity of these compounds to the diphenhydramines. The antihistaminic activity is very much less than the anticholinergic activity but a similar lack of stereoselectivity appears.

Table IV. pD_2 and Intrinsic Activity Values for Agonist Activity of 1,3-Dioxolanes at the Muscarinic Receptor



No.	R	R'	Guinea pig long muscle I act.[a]	Guinea pig long muscle pD_2[b] ± S. E.	π
19	CH_3	CH_3	1.0	4.87 ± 0.03 (4)	1.04
20	C_2H_5	C_2H_5	0.85	4.89 ± 0.02 (4)	1.94
21	$CH(CH_3)_2$	$CH(CH_3)_2$	0.68	4.44 ± 0.02 (6)	2.60
22	Cyclopropyl		0.67	4.57 ± 0.03 (5)	2.08
23	CH_3	H	1.0	7.61 ± 0.02 (19)	0.52

[a] Intrinsic activity (I act.) is a measure of the maximum response produced by an agonist relative to *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide. [b] pD_2 values are $-\log ED_{50}$ of the agonist in question.

It has long been established that agonist → antagonist transitions can take place by progressive incorporation of nonpolar groups. More quantitative expressions of this fact correlate antagonist activity with the π substituent constant derived from octanol-H_2O partition data[14,19a] and which is some measure of hydrophobicity. Such correlations are becoming increasingly common.[1,14,19a,b] Pratesi, *et al.*,[20] observed a pA_2-π correlation for the anticholinergic activities of a series of *N*-alkyl-*N*-phenyl-2-aminoethyl-(*N*-piperidino)ethiodides deviating from linearity with large alkyl groups (>C_5H_{11}) that had presumably exceeded the

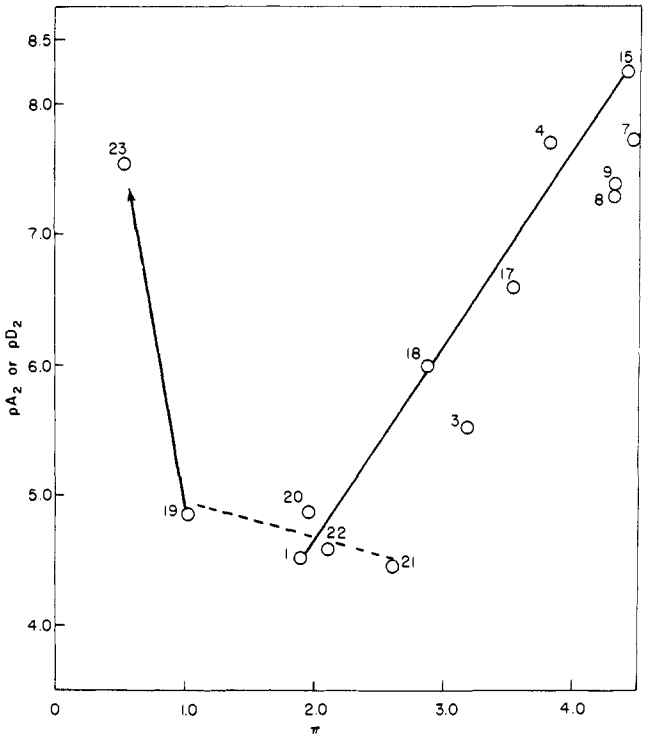


Figure 1. A plot of π against pA_2 (antagonists) or pD_2 (agonists) values. Numbers refer to compounds listed in Tables II and IV. Full line (antagonists) represents regression eq 2 and the dashed line (agonists), regression eq 3.

size of the nonpolar binding area. A similar correlation can be derived[1] from data from a series of quaternary alkarylammonium compounds studied by Abramson, *et al.*[21]

The transition from agonist to antagonist in the 1,3-dioxolanes noted previously[22] can be seen from a comparison of Tables II and IV. Figure 1 is a plot of π *vs.* pA_2 and pD_2 values. Not all of the compounds listed in Table II have been included: of the series **10–15** only **15** has been plotted on the basis that the differences in the series were quite small but that **15** represented the optimum interaction. Similarly only **1** and **4** were plotted. The 2,2-dicyclohexyl compound (**16**) is not plotted on Figure 1: its activity clearly represents an extreme deviation from the π-pA_2 relationship shown for the other dioxolanes. Possibly this substituent has exceeded the binding capacity of the nonpolar area. Regression analysis with the 9 antagonists (**1**, **3**, **4**, **7–9**, **15**, **17**, **18**) shown in Figure 1 yields

$$pA_2 = 1.304\pi + 2.059 \ (n = 9, r = 0.942, r^2 = 0.887) \qquad (1)$$

and with the exclusion of **3**, **7**, **8**, and **9**, for which steric interactions are almost certainly of importance, the correlation is significantly better

$$pA_2 = 1.467\pi + 1.789 \ (n = 5, r = 0.987, r^2 = 0.971). \qquad (2)$$

An attempt to correlate similarly the pD_2 values of the agonists listed in Table IV was strikingly less successful. The agonist and partial agonist activity of the four 2,2-disubstituted 1,3-dioxolanes (**19–22**) is relatively independent of π and the correlation is poor

$$pD_2 = 0.27\pi + 5.21 \ (n = 4, r = 0.79, r^2 = 0.62). \qquad (3)$$

A precise interpretation of the linear free energy relationships shown in Figure 1 is impossible to offer since the pA_2 and pD_2 values that are being correlated with π represent two different experimental quantities: the pA_2 values are probably a measure of binding only but the pD_2 values include not only affinity but also some measure of the ability of the ligand to initiate the observed response. Nevertheless, the rather satisfactory pA_2-π correlation for the antagonists extending over a 10^4 range of activity does emphasize the dominant role of the nonpolar interaction in determining antagonist activity. It is also clear that the molecular parameters determining agonist activity are substantially different. The vigorous deviation of the highly potent agonist **23** from the regression line described by eq 3 serves to reemphasize the well-known highly stereoselective and structurally demanding requirements for high agonist activity in this receptor system.[1] The uniqueness of structure **23** was also noted by Belleau and Lavoie[23] in their thermodynamic analysis of ligand binding to AChE.[23] The interaction of **23** differs from the other 4 agonists (**19–22**) whose significantly lower activity is relatively independent of π. However, the dimensions of π alone do not uniquely determine agonist or antagonist activity for the π values of **20–22** exceed those of **1** yet the latter exhibits only antagonist activity: apparently, the flexibility of the nonpolar substituents in the former may be of importance in this regard.

Hence, the linear free energy relationships defined in Figure 1 may define transitions in binding patterns for agonists and antagonists. There exists a highly stereoselective and restricted binding of potent agonists (**23**) at a relatively polar site: this is the neurotransmitter recognition site proper and accommodates ligands (ACh, Ac-β-Me-Ch, muscarine) of closely related sturcture.[1] With progressive nonpolar character (**19–22**) interaction may become progressively more difficult and less effective at this site, but the flexibility of the 2 substituents may ensure that some interaction is still possible. The incorporation of the rigid 2-aryl or 2-cyclohexyl substituents (**1–16**) precludes interaction at this site and serves to reorient ligand binding to a dominantly nonpolar area. It is not possible to state from these results alone whether the agonist or antagonist binding sites are totally distinct or whether they are only partially distinct retaining a common binding area (ammonium binding site). Other lines of evidence[1,15] would appear to support the latter conclusion.

Acknowledgments. This work was supported in part by a grant from the National Institutes of Health (NS 09573). We thank the American Pharmaceutical Association for the award (1968-1969) of a Mead-Johnson Undergraduate Research Grant (R. C. D. and D. J. T.).

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Medicinal Chemical Studies on Antiplasmin Drugs. 4. Chemical Modification of *trans*-4-Aminomethylcyclohexanecarboxylic Acid and Its Effect on Antiplasmin Activity†

Atsuji Okano, Masato Inaoka, Shoichi Funabashi, Masahiro Iwamoto, Sumiro Isoda, Reimei Moroi, Yasushi Abiko, and Miyoshi Hirata.*

Research Laboratories, Daiichi Seiyaku Company, Ltd., Tokyo, Japan. *Received July 12, 1971*

A series of N-substituted derivatives, amides, and esters of *trans*-4-aminomethylcyclohexanecarboxylic acid (*trans*-AMCHA) were synthesized and evaluated for their antiplasmin activity. Among those, Ph ester derivatives were found to be superior to *trans*-AMCHA. In particular, a high order of the activity was achieved with para-substituted Ph esters. This paper reports the synthetic method, the antiplasmin activity, and the structure-activity relationship.

Several synthetic inhibitors of plasmin have been reported, including ε-aminocaproic acid (EACA), p-aminomethylbenzoic acid (PAMBA), *trans*-4-aminomethylcyclohexanecarboxylic acid (*trans*-AMCHA), and 4-aminomethylbicyclo[2,2,2]octanecarboxylic acid. Some of them have been subjected to chemical modifications in a search for a new inhibitor. Nagamatsu, *et al.*[1], reported the inhibitory effects of various N-substituted compounds of L-lysine and esters of EACA on plasmin activity, and Muramatsu, *et al.*[2-6] described the extensive inhibitory effect of various esters on plasmin and trypsin activities and the relationship between their chemical structure and the inhibitory effect. Among the various saturated aliphatic esters of EACA, the *n*-hexyl ester showed the most extensive inhibitory effect, while branching of the alkyl chain resulted in a decrease of this effect. Markwardt[7-9] and his coworkers synthesized various PAMBA derivatives and studied the relationship between chemical structure and antiproteolytic activity of these compounds, and they demonstrated that the benzyl esters were most potent. Modification of *trans*-AMCHA had been limited to hexyl[6,10] and *p*-nitrophenyl[11] esters. The preceding paper[12] from our laboratories indicated that introduction of Me into the cyclohexane ring or the side chain of *trans*-AMCHA resulted in a decrease of the antifibrinolytic activity.

Recently, however, Muramatsu and Fujii[13] observed the excellent inhibitory effects of Ph ester and *p*-carboxyethylphenyl ester of *trans*-AMCHA on plasmin, trypsin, plasma kallikrein, and thrombin. The present paper deals with the relationship between the antiplasmin activity and the chemical structure of ester derivatives of *trans*-AMCHA including these Ph esters. Other chemical modifications of *trans*-AMCHA, N-substitution and amidation, are also described here.

Chemistry. *trans*-AMCHA derivatives used in this study were synthesized mainly according to the methods A-J described in the Experimental Section, and are shown in Tables I and II. Most of these methods were used widely to obtain N-substituted amide and ester derivatives of the amino acid. Carbobenzoxy (Cbz) *trans*-AMCHA and its acid chloride were found very useful for the preparation of *trans*-AMCHA derivatives. Physical properties of Cbz intermediates are tabulated in Table III.

Structure-Activity Relationships. The substances listed in Tables I and II were examined for their antiplasmin activity in the caseinolytic and fibrinolytic reactions using *trans*-AMCHA, its benzyl ester (**63**), or its phenyl ester (**75**) as reference standards.

From the data in Table I, it was apparent that introduction of substituent groups into the aminomethyl moiety or amidation of *trans*-AMCHA caused a drastic decrease in the antiplasmin activity with only one exception (**14**).

As shown in Table II, the antiplasmin activity of a series of alkyl esters (**35-46**) was somewhat superior to that of *trans*-AMCHA in caseinolysis, and the relationship between the activity and the length of the ester moiety was in good agreement with the result of the EACA ester investigated earlier,[1-6] that is, the *n*-hexyl ester was found to be the most active agent in this series and the activity of the unbranched ester (**37,39**) was greater than that of the branched chain compd with the same number of C atoms (**38,40,41**). Furthermore, it was very interesting to find that the unsaturated alkyl esters (**54,55**) having a double or triple bond at the β position of the alkoxy group were more potent than the corresponding saturated alkyl ester (**37**).

The potency of the benzyl ester (**63**) relative to *trans*-

†Presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.