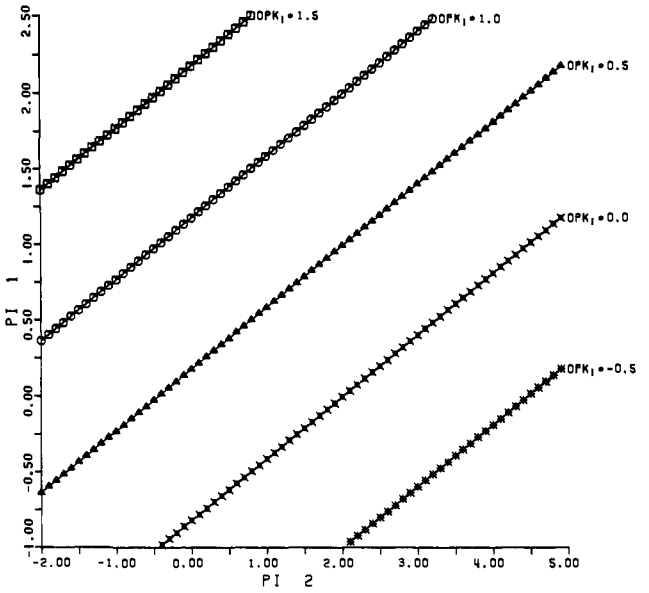

Figure 2. Diagram of isoergs.

substituents hydrophilic or only very slightly lipophilic.

In order to check this statement, several 3,5-dichloro-2,6-dihydroxybenzoic acid anilides were synthesized and tested (Table III). By incorporating the new derivatives into the overall correlation we obtained

$$pK_i\text{-R} = 0.883\ (\pm 0.066)\pi_1 + 0.688\ (\pm 0.039)\pi_2 + 4.645\ (\pm 0.088) \quad (4)$$

$$n = 30;\ s = 0.219;\ r = 0.963;\ F_{2,27} = 173.08;\ p < 0.001$$

$$pK_i\text{-L} = 1.371\ (\pm 0.123)\pi_1 + 0.472\ (\pm 0.068)\pi_2 + 5.080\ (\pm 0.148) \quad (5)$$

$$n = 32;\ s = 0.449;\ r = 0.908;\ F_{2,29} = 67.92;\ p < 0.001$$

A comparison of these equations with those originally obtained shows that the new derivatives can be incorporated well into the existing scheme.

The correlation of ΔpK_i with π_1 and π_2 was also repeated with the inclusion of the new derivatives and yielded the improved equation

$$\Delta pK_i = 0.501\ (\pm 0.137)\pi_1 - 0.204\ (\pm 0.080)\pi_2 + 0.411\ (\pm 0.181) \quad (6)$$

$$n = 30;\ s = 0.453;\ r = 0.765;\ F_{2,27} = 19.04;\ p < 0.001$$

For this equation a diagram of isoergs† was plotted, which permits us to read ΔpK_i for each desired parameter combination (Figure 2). All points on one of the straight lines (isoergs) have the same (ideal) chemotherapeutic index. Below the straight line for $\Delta pK_i = 0$ the toxicity is greater for the host organism than for the parasite. The farther one goes up to the left from this critical line, the more favorable the ratio between the two inhibition constants becomes, and one sees that a combination of a high π_1 value and a negative π_2 value will provide the best results.‡

References

(1) G. Lämmler and H. Loewe, *Arzneim.-Forsch.*, 12, 15, 164 (1962).
(2) J. C. Boray, *Advan. Parasitol.*, 7, 95 (1969).
(3) H. Metzger and D. Düwel, *Int. J. Biochem.*, in press.
(4) H. Metzger, *Z. Parasitenk.*, 34, 271 (1970).
(5) L. W. de Zoeten and K. Tipker, *Hoppe Seyler's Z. Physiol. Chem.*, 350, 691 (1969).
(6) R. K. Prichard and P. J. Schofield, *Comp. Biochem. Physiol.*, 25, 1005 (1968).
(7) D. Düwel and H. Metzger, *J. Med. Chem.*, 16, 433 (1973).
(8) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, 86, 5175 (1964); C. Hansch and S. M. Anderson, *J. Org. Chem.*, 32, 2583 (1967).
(9) H. H. Jaffé, *Chem. Rev.*, 53, 191 (1953).
(10) R. W. Taft in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 556.

†By analogy with the terms "isotherms" or "isobars," we suggest the term "isoergs" for lines denoting the same biological response.

‡After the work for this manuscript had been completed, some additional compounds were synthesized and tested in the enzyme assay. They are listed in the preceding paper with their K_i values. Some of them could not be used here, either because of structural changes or because of missing substituent parameters. But there are still some 20 congeners of this series that can be included. However, since their activity could be predicted reasonably well with the existing regression equations, we did not think it necessary to include them in the Hansch analysis.

Studies on the Stereospecificity of Closely Related Compounds Which Block Postganglionic Acetylcholine Receptors in the Guinea-Pig Ileum

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Esters of dimethylamino- diethylamino-, pyrrolidino-, and piperidinoethanols have been prepared with the resolved forms of mandelic, cyclohexylphenylacetic, cyclohexylphenylglycolic, and α-methyltropic acids and converted to their hydrochlorides, methiodides, and ethiodides. Enantiomeric pairs of quaternary derivatives of hyoscyamine, hyoscine, and homatropine have also been made and the affinity constants of all the compounds have been measured for the postganglionic acetylcholine receptors of the guinea-pig ileum. The derivatives of mandelic and cyclohexylphenylacetic acids had only low stereospecificity but those of cyclohexylphenylglycolic and α-methyltropic acids had considerable stereospecificity as did the derivatives of hyoscyamine and hyoscine. Even though the asymmetric center is at the other end of the molecule, changes in the composition of the onium group produce considerable changes in stereospecificity in these series of enantiomers and possible reasons for this are discussed.

Relationships between chemical structure and binding to acetylcholine receptors have been investigated by Abramson, Barlow, Mustafa, and Stephenson[1] who measured the affinities of over 100 compounds related to acetylcholine for the postganglionic acetylcholine receptors of the guinea-pig ileum. From the results they concluded that any change in structure which could lead to increased affinity, such as an increase in size, was likely to lead also to a disturbance in

the binding of the rest of the molecule. It appeared that changes at one end of a molecule produced surprisingly large disturbances in the binding of the other end and it therefore seemed worthwhile investigating what happened when the other end contained an asymmetric center.

The compounds made and tested had the general formula $RCOOCH_2CH_2R'$, where the series of onium groups R' was $^+NMe_2H$, $^+NMe_3$, $^+NMe_2Et$, $^+NEt_2H$, $^+NEt_2Me$, $^+NEt_3$, $HN^+(CH_2)_3CH_2$, $MeN^+(CH_2)_3CH_2$, $EtN^+(CH_2)_3CH_2$, $HN^+(CH_2)_4CH_2$, $MeN^+(CH_2)_4CH_2$, and $EtN^+(CH_2)_4CH_2$ and the acid portion, R, was mandelyl, cyclohexylphenylacetyl, cyclohexylphenylglycololyl, and α-methyltropyl. We have also made and tested resolved forms of homatropine, and their methyl and ethyl iodides, and of hyoscine and hyoscyamine and their methyl, ethyl, *n*-propyl, and *n*-butyl iodides.

If the change in the composition of the onium group produces different effects on the binding of the two enantiomers, it should affect the stereospecific index, but it was not clear from the literature to what extent the stereospecific index might be expected to vary in series such as these, where the changes in biological activity are due only to changes in affinity. Some short series of enantiomeric pairs of atropine-like compounds have been studied[2-5] but only a few[4-6] have been tested in conditions in which they have had time to come into equilibrium with the receptors. A further complication is that the enantiomers should all have the same stereochemical purity. It is not satisfactory to obtain the enantiomers by resolution of a racemate. With highly stereospecific pairs very small differences in stereochemical purity markedly affect the biological results[7] and, even if resolution is complete, it is almost impossible to show that this is so. To study changes in stereospecificity the series of compounds should be obtained from the same resolved source by methods which do not involve racemization. Few results have been obtained in this way.[5]

In the present work all measurements of affinity were made in conditions in which the compounds were allowed time to come into equilibrium with the receptors and the series of enantiomeric pairs were all obtained from common resolved sources. The extent to which racemization may have occurred is considered below (see Results).

Experimental Section

Chemistry. Melting points were measured with a Mettler FP 1 instrument, connected to a Vitatron pen recorder; the rate of heating was usually 0.2°/min. In the resolution of the acids some rotations were measured with a Bellingham and Stanley Model D polarimeter but those of all final products were measured with a Model B spectropolarimeter with a 5-cm cell fitted with silica windows. The final products were usually checked for chromatographic homogeneity on paper in 1-butanol–ethanol–water (5:5:2), developed with a modified Dragendorff reagent,[8] and were analyzed for ionized halide gravimetrically with samples of 50–150 mg.

Resolved Acids. (*R*)- and (*S*)-mandelic acids were bought from Fluka A. G. and had mp 130–133°, $\alpha^{21}D$ −158 and +159°, respectively (*c* 2, water).

(+)- and (−)-Cyclohexylphenylacetic Acids. The racemic acid[9] (170 g) was added to quinine (252 g) dissolved in boiling ethanol and left at +4° for 2 hr. The salt which crystallized was filtered off, and after eight recrystallizations from ethanol had a constant mp 170.8–171.4° and rotation $\alpha^{25}D$ +11.05° (*c* 20, chloroform). This was treated with excess sulfuric acid (0.5 *M*) and extracted with ether. The extract was dried with anhydrous magnesium sulfate, and the crude (+)-acid recrystallized from petroleum ether: mp 100–102°; $\alpha^{25}D$ +38.7° (*c* 20, chloroform).

The mother liquors, left after filtration of the quinine salt, were combined, concentrated, converted back to the acid, and added to quinidine, dissolved in boiling ethyl acetate. The hot solution was filtered and left at +4° overnight. The salt which crystallized was filtered off and after four crystallizations had a constant mp 133.4–134.4° and rotation $\alpha^{25}D$ +48.10° (*c* 20, chloroform). This was converted to the (−)-acid which had mp 100–101°, $\alpha^{25}D$ −38.8° (*c* 20, chloroform). The rotations of the quinine and quinidine salts were very temperature-sensitive; αD varied as much as 1°/°C.

(*R*)- and (*S*)-Cyclohexylphenylglycolic Acids. The racemic acid[10] was resolved with quinine;[11] after six recrystallizations from ethanol the quinine salt of the (*S*)-(+)-acid had constant mp 222.4–222.6° dec and rotation $\alpha^{25}D$ −76.0° (*c* 4.0, chloroform) and when converted to the acid this had mp 140.6–141.1°, $\alpha^{25}D$ +24.8° (*c* 4.5, ethanol). The mother liquors were combined, concentrated, converted back to the acid, dissolved in ethanol, added to (−)-ephedrine dissolved in aqueous ethanol, and left at +4° overnight. The salt which crystallized was filtered off, and after four recrystallizations from aqueous ethanol had constant mp 170.8–171.6° and rotation $\alpha^{25}D$ −43.5° (*c* 2, ethanol). When converted to the acid this had mp 141.9–142.3°, $\alpha^{25}D$ −24.9° (*c* 4.5, ethanol). The values in the literature[11] for the (+)- and (−)-acids respectively are mp 142–143 and 145°; αD +25.8 ± 1 and −25.2 ± 1° (*c* 4.48, ethanol). This acid has also been resolved with (+)- and (−)-amphetamine by Ellenbroek.[12] The assignment of the *R* configuration to the (−)-acid follows from its synthesis by a stereospecific route.[13]

(+)- and (−)-α-Methyltropic Acids. The racemic acid was prepared by the method of Vecchi and Melone,[14] but Professor G. Maffii and Professor G. Nathanson of Lepetit S. P. A. very kindly provided us with quantities of the resolved forms: (+)-α-methyltropic acid had mp 86–87°; αD +25.3°; (−)-α-methyltropic acid had mp 85–86°; αD −27.0°.

Resolved Bases. (*R*)- and (*S*)-Hyoscyamine. The *S* isomer was bought as the hydrobromide from Macfarlan Smith Ltd. and converted to base, mp 103.8–104.6°, $\alpha^{23}D$ −24.0° (*c* 5, 50% aqeous ethanol). The *R* isomer was obtained from atropine by resolution with camphor-10-sulfonic acid[15] and had mp 107.2–108.2°, $\alpha^{26}D$ +21.4° (*c* 5, 50% aqueous ethanol). The values in the literature[15] for (*S*)- and (*R*)-hyoscyamine respectively are mp 108–109 and 105–106°; $\alpha^{20}D$ −21 and +21° (*c* 0.173, 50% aqueous ethanol).

(*R*)- and (*S*)-Hyoscine. The *S* isomer was bought as the hydrobromide from British Drug Houses Ltd. and the *R* isomer was kindly given us by Dr. R. P. Paton of Macfarlan Smith Ltd. Hyoscine base could only be obtained from these in the form of a syrup.

Esters. The procedure of Miescher and Hoffman[16] was used to prepare the esters of the resolved forms of cyclohexylphenylglycolic and α-methyltropic acids and that of Burtner and Cusic[17] was used to prepare the esters of cyclohexylphenylacetic and mandelic acids. The esters with mandelic acid decomposed on distillation and we failed to obtain the dimethylaminoethyl ester at all. The esters of the resolved forms of mandelic acid with tropine (the enantiomeric forms of homatropine) were prepared by passing dry hydrogen chloride through a mixture of the acid and tropine heated to 110°, as described by Jowett and Pyman[18] for the synthesis of a number of esters of tropine.

Quaternary Salts and Other Derivatives. Quaternary salts were usually prepared by dissolving the tertiary base in ethyl methyl ketone, adding excess methyl or ethyl iodide, and leaving the solution for 2 days. If no crystals had formed, the solution was heated under reflux for 2 hr and ethyl acetate was added until it became turbid. For the alkylation of hyoscyamine and hyoscine, acetonitrile was used as solvent and with the larger groups it was necessary to heat the mixture under reflux for up to 18 hr.

Most of the quaternary salts were recrystallized from ethyl methyl ketone but with some it was necessary to add ethanol and with others ethyl acetate.

Hydrochlorides of the teriary bases were prepared by blowing dry hydrogen chloride through a solution of the base in ethanol.

The compounds prepared are listed in Table I, together with their melting points and molar rotations. Except where stated, all gave satisfactory analyses for ionized halide (see above).

Pharmacology. Estimates of the logarithm of the affinity constants of the compounds for the postganglionic acetylcholine receptors of the guinea-pig ileum were made by methods already described.[1,19,20] The ileum was suspended in aerated Tyrode's solution containing hexamethonium, 2.75×10^{-4} *M*, maintained at 37°, with carbachol as the agonist, applied for 30 sec (then washed out) with an interval of 90 sec between each application. When control responses to alternate high and low concentrations of carbachol were steady, the preparation was exposed continuously to a concentration of the antagonist, present in the Tyrode's solution for washing the tissue and in the Tyrode's solution containing the concentrations of carbachol. These concentrations of carbachol were increased so that, after the effect of the antagonist had been seen to become established,

Table I. Melting Points, Molar Rotations, Values of Log K for Acetylcholine Receptors, and Log Stereospecific Index (Log SSI)[a]

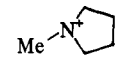
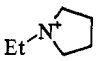
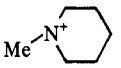
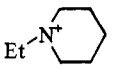
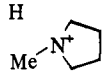
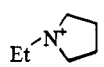
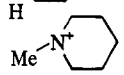
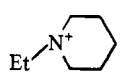
		Mp, °C	M 589 nm	M 300 nm	Log K	Log SSI
			A. PhCH(OH)COOCH$_2$CH$_2$-[b]			
$^+NMe_3$		± 112–116			5.288 ± 0.069 (7)	
$^+NMe_2Et$		± 85–88			5.893 ± 0.027 (7)	
$^+NEt_2Me$		*S* 92–97	+159	+1416	6.044 ± 0.030 (12)	
		R 92–98	−157	−1437	6.153 ± 0.055 (7)	0.109
		± 122–123			5.979 ± 0.022 (7)	
					(6.102)	
$^+NEt_3$		*S* 121–124	+154	+1347	6.117 ± 0.022 (8)	
		R 118–121	−151	−1321	6.103 ± 0.097 (9)	$\bar{1}$.986
		± 105–106			6.086 ± 0.022 (9)	
					(6.110)	
		*S* 107–109	+166	+1471	5.597 ± 0.031 (7)	
		R 107–109	−161	−1414	5.974 ± 0.014 (5)	0.377
		± 103–106			5.902 ± 0.024 (8)	
					(5.825)	
		*S* 96–99	+161	+1377	5.911 ± 0.036 (7)	
		R 96–100	−158	−1376	6.368 ± 0.025 (8)	0.457
		± 86–89			6.233 ± 0.015 (11)	
					(6.197)	
		*S* 109–111	+174	+1535	5.321 ± 0.039 (5)	
		R 104–108	−163	−1421	5.674 ± 0.009 (5)	0.353
		±89–93			5.627 ± 0.012 (6)	
					(5.533)	
		*S* 110–115	+162	+1356	5.501 ± 0.010 (7)	
		R 110–115	−161	−1396	6.021 ± 0.065 (6)	0.520
		± 99–101			5.818 ± 0.050 (8)	
					(5.835)	
			B. 			
$^+NMe_2H$	+	138.8–139.6	+102	+986	7.392 ± 0.040 (8)	0.560
	−	138.6–139.8	−100	−981	7.952 ± 0.064 (6)	
$^+NMe_3$	+	160.2–161.4	+121	+1137	8.100 ± 0.033 (6)	0.474
	−	161.0–161.7	−120	−1128	8.574 ± 0.010 (8)	
	±	(bromide)			8.438	
					(8.399)	
$^+NMe_2Et$	+	125.2–126.0	+114	+1115	8.500 ± 0.038 (8)	0.492
	−	124.6–125.4	−114	−1122	8.992 ± 0.024 (6)	
	±	(bromide)			8.970	
					(8.812)	
$^+NEt_2H$	+	146.6–147.2	+96	+1006	8.000 ± 0.020 (6)	0.216
	−	146.0–147.0	−98	−1009	8.216 ± 0.043 (6)	
$^+NEt_2Me$	+	111.0–111.7	+105	+1081	8.490 ± 0.032 (6)	0.359
	−	111.0–111.7	−105	−1070	8.849 ± 0.049 (7)	
	±	(bromide)			8.699	
					(8.705)	
$^+NEt_3$	+	153.6–154.0	+99	+1038	8.431 ± 0.016 (4)	0.102
	−	153.9–154.3	−97	−1047	8.533 ± 0.016 (7)	
	±	(bromide)			8.566	
					(8.485)	
	+	153.6–154.6	Not measured		7.881 ± 0.032 (6)	0.381
	−	154.9–155.5			8.262 ± 0.020 (6)	
	+	87.2–88.2	+104	+1062	8.301 ± 0.023 (7)	0.374
	−	88.2–90.2	−103	−1066	8.675 ± 0.027 (6)	
	±	(bromide)			8.526	
					(8.527)	
	+	127.2–128.3	+101	+1052	8.568 ± 0.020 (6)	0.367
	−	129.0–129.6	−102	−1060	8.935 ± 0.025 (7)	
	±	(bromide)			8.677	
					(8.789)	
	+	195.7–196.4	+106	+1098	8.427 ± 0.021 (6)	$\bar{1}$.688
	−	196.1–196.8	−105	−1074	8.115 ± 0.019 (8)	
	+	146.4–148.0	+101	+1074	8.301 ± 0.016 (6)	0.002
	−	146.5–148.0	−101	−1075	8.303 ± 0.013 (7)	
	±	(bromide)			8.290	
					(8.302)	
	+	124.0–125.2	+93	+1013	8.224 ± 0.050 (7)	$\bar{1}$.927
	−	124.6–125.6	−94	−1030	8.151 ± 0.034 (6)	
	±	169–170			8.099	
					(8.188)	

Table I *(Continued)*

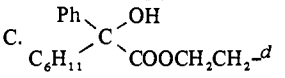
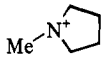
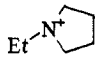
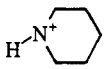
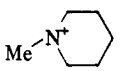
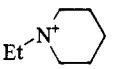
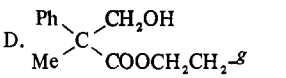
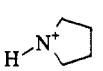
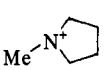
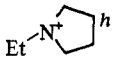
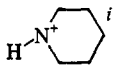
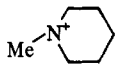
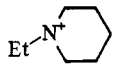
		Mp, °C	M 589 nm	M 300 nm	Log K	Log SSI
		C. 				
$^{+}NMe_2H$[e]	*S*	197.0–197.4	−20	+26	6.977 ± 0.046 (7)	1.889
	R	197.4–197.7	+17	−28	8.866 ± 0.067 (11)	
$^{+}NMe_3$[f]	*S*	141.1–142.3	−23	+148	7.257 ± 0.021 (13)	2.390
	R	141.6–142.4	+24	−150	9.647 ± 0.073 (11)	
	±	166.9–167.3			9.365 (9.348)	
$^{+}NMe_2Et$	*S*	165.2–165.8	−26	+135	7.882 ± 0.019 (7)	2.158
	R	165.0–165.9	+25	−132	10.040 ± 0.039 (8)	
	±	124.1–124.6			9.804 (9.742)	
$^{+}NEt_2H$	*S*	221.2–221.5	−14	+43	7.756 ± 0.053 (7)	1.543
	R	220.4–220.6	+13	−45	9.299 ± 0.054 (5)	
$^{+}NEt_2Me$	*S*	153.1–153.6	−26	+107	8.150 ± 0.036 (5)	1.850
	R	152.6–153.1	+26	−107	10.000 ± 0.039 (7)	
	±	136.5–137.0			9.777 (9.705)	
$^{+}NEt_3$	*S*	197.2–197.4 dec	−29	+66	7.989 ± 0.035 (7)	1.611
	R	196.5–196.7 dec	+28	−66	9.600 ± 0.068 (8)	
	±	175.7–176.2			9.482 (9.310)	
	*S*	207.2–207.4	−17	+33	7.191 ± 0.025 (6)	1.669
	R	206.3–206.5	+17	−31	8.860 ± 0.056 (10)	
	*S*	192.2–192.8	−25	+123	7.488 ± 0.046 (6)	2.147
	R	182.3–183.1	+26	−117	9.635 ± 0.047 (6)	
	±	173.4–174.0			9.473 (9.337)	
	*S*	140.0–140.8	−26	+83	7.765 ± 0.059 (8)	1.958
	R	Sinters 140	+27	−78	9.723 ± 0.024 (5)	
	±	136.8–137.3			9.588 (9.427)	
	*S*	224.2–224.4 dec	−17	+78	6.935 ± 0.048 (8)	2.073
	R	223.4–223.6 dec	+15	−76	9.008 ± 0.071 (9)	
	*S*	186.5–187.4	−28	+119	7.334 ± 0.033 (6)	2.140
	R	186.9–187.3	+27	−117	9.474 ± 0.041 (6)	
	±	183.6–184.1			9.215 (9.176)	
	*S*	172.2–173.2	−26	+93	7.413 ± 0.013 (6)	1.840
	R	172.2–173.2	+25	−100	9.253 ± 0.037 (8)	
	±	184.6–184.8			9.801 (8.958)	
		D. 				
$^{+}NMe_2H$	+	77.5–79.0	+27.6	+247	5.570 ± 0.031 (5)	1.784
	−	76.2–80.0	−30.0	−247	7.354 ± 0.003 (6)	
	±	78.2–80.0				
$^{+}NMe_3$	+	116.4–117.9	+4.8	+32.4	6.058 ± 0.018 (7)	2.061
	−	117.1–118.4	−4.8	−36.4	8.119 ± 0.035 (8)	
	±	128.0–129.2	(in MeOH)			
$^{+}NMe_2Et$	+	131.0–131.7	+36.0	+316	6.648 ± 0.061 (6)	2.193
	−	128.2–130.2	−35.2	−302	8.841 ± 0.031 (8)	
	±	115.0–116.0				
$^{+}NEt_2H$	+	92.2–93.7	+31.2	+262	6.126 ± 0.050 (7)	1.870
	−	93.5–95.5	−31.2	−256	7.996 ± 0.016 (5)	
	±	115.8–117.5				
$^{+}NEt_2Me$	+	124.8–127.4	+38.0	+332	6.681 ± 0.030 (7)	1.797
	−	125.5–127.5	−38.8	−328	8.478 ± 0.037 (7)	
	±	94.0–95.6				
$^{+}NEt_3$	+	171.2–172.9	+43.2	+358	6.607 ± 0.031 (7)	1.679
	−	172.1–173.4	−44.4	−360	8.286 ± 0.016 (8)	
	±	155.8–156.2				
	+	89.0–90.6	+29.2	+254	5.126 ± 0.039 (5)	2.519
	−	85.0–87.5	−30.0	−256	7.645 ± 0.038 (6)	
	±	74.4–76.2				
	+	99.0–100.5	+32.0	+296	6.564 ± 0.006 (5)	1.665
	−	97.9–99.4	−34.4	−291	8.229 ± 0.026 (7)	
	±	93.2–94.5				

Table I *(Continued)*

		Mp, °C	*M* 589 nm	*M* 300 nm	Log *K*		Log SSI
	+	Could not be	+34.4	+304	6.739 ± 0.035	(7)	1.550
	−	crystallized	−35.2	−289	8.289 ± 0.020	(6)	
	±	(bromide, 111-112)					
	+	141.8-143.0	+26.8	+242	5.312 ± 0.037	(6)	2.140
	−	126.0-126.8	−27.2	−233	7.452 ± 0.026	(7)	
	±	132.0-134.5					
	+	144.5-146.3	+33.2	+294	6.406 ± 0.022	(5)	1.693
	−	143.5-145.5	−34.0	−289	8.099 ± 0.037	(7)	
	±	137.2-138.5					
	+	102.0-103.0	+37.6	+328	6.174 ± 0.034	(6)	2.290
	−	102.0-103.3	−38.8	−331	8.464 ± 0.039	(6)	
	±	97.0-98.3					
			E. Hyoscyamine Derivatives[j]				
HBr	*S*	148.6-151.9	−96	−991	9.380 ± 0.029	(5)	2.519
	R	150.4-151.4	+97	+995	6.861 ± 0.071	(8)	
	±	(sulfate)			9.007		
					(9.080)		
MeI	*S*	186.1-186.6	−93	−979	9.666 ± 0.064	(10)	1.941
	R	185.5-185.9	+94	+994	7.725 ± 0.052	(9)	
	±	174.5-176.0			9.454 ± 0.013	(4)	
					(9.370)		
EtI	*S*	152.2-153.3	−89	−946	8.787 ± 0.040	(6)	1.726
	R	161.8-163.2	+88	+912	7.061 ± 0.040	(6)	
	±	166.8-167.9[k]			8.239 ± 0.022	(5)	
					(8.494)		
PrI	*S*	173.2-175.8	−79	−877	7.500 ± 0.040	(8)	1.237
	R	168.0-170.0	+81	+869	6.263 ± 0.058	(6)	
	±	sinters 159-164			7.244 ± 0.022	(6)	
					(7.224)		
BuI	*S*	152.6-155.5	−79	−887	7.087 ± 0.013	(6)	1.202
	R	147.0-150.0	+80	+861	5.885 ± 0.018	(6)	
			F. Hyoscine Derivatives[l]				
HBr	*S*	195.1-195.5	−114	−1190	9.363 ± 0.062	(6)	1.791
	R	170-190[m]	+186	+1218	7.572 ± 0.015	(12)	
MeI	*S*	220.7-221.0	−109	−1184	9.702 ± 0.068	(9)	1.080
	R	210.0-210.9	+103	+1105	8.622 ± 0.038	(6)	
EtI	*S*	185.7-186.0	−108	−1179	8.603 ± 0.055	(5)	1.450
	R	182.0-182.8	+104	+1147	7.153 ± 0.036	(7)	
PrI	*S*	160.3-160.8	−103	−1137	8.269 ± 0.048	(6)	1.499
	R	157.1-157.4	+99	+1107	6.770 ± 0.045	(6)	
BuI	*S*	86.0-93.0	−96	−1078	7.162 ± 0.059	(7)	
			G. Homatropine Derivatives[n,o]				
Sulfate	*S*	232.7-232.8	+404		6.991 ± 0.025	(7)	0.450
	R	225.7-225.9	−417		7.441 ± 0.031	(7)	
MeI	*S*	210-214	+154		7.279 ± 0.017	(5)	0.581
	R	216-220	−175	−1737	7.860 ± 0.023	(6)	
EtI	*S*	200-202	+175	+1653	6.774 ± 0.028	(6)	0.194
	R	202-204	−191	−1888	6.968 ± 0.027	(8)	

[a]Values of log *K* are shown with their standard errors and the number of estimates. Values for the racemate calculated from those for the enantiomers are shown in parentheses. Melting points and results for the racemates in parts B and C are given by Abramson, *et al.*[1] Unless otherwise stated, all quaternary salts are iodides and tertiary bases are hydrochlorides and gave acceptable analyses for halide ion. [b]Molar rotations at *c* 2 × 10^{-2} *M* (EtOH). [c]Molar rotations at *c* 5 × 10^{-2} *M* (EtOH). [d]Molar rotations at *c* 5 × 10^{-2} *M* (MeOH). [e]Brimblecombe, *et al.*,[5] recorded log *K* 7.07 and 9.06. [f]Brimblecombe, *et al.*,[5] recorded log *K* 7.38 and 9.66. [g]Molar rotations at *c* 5 × 10^{-2} *M* (water). [h]The racemic form of this compound was obtained satisfactorily as the bromide (the iodide would not crystallize) but the enantiomeric forms of the bromide would not crystallize and were not analyzed, though the rotations and biological activity were estimated with the dried syrup. [i]We have no explanation for the difference between the melting points of these enantiomers; the rotations and analyses were satisfactory and the bases from which they were formed had the same bp 140-142° (4 × 10^{-4} mm). [j]Molar rotations at *c* 4 × 10^{-2} *M* (water). [k]With a different sample of purified atropine ethiodide and in a quite separate set of experiments (2 years previously) we obtained a value of 8.198 ± 0.016 (4). [l]Molar rotations at *c* 2 × 10^{-2} *M* (water). [m]The sample of (+)-hyoscine hydrobromide was probably contaminated with traces of optically active acid used in its resolution; the derivatives made from it had rotations reasonably close to those of their *S* enantiomers. There was insufficient material for analysis of the *R* compounds but the *S* compounds gave acceptable analyses for halide. [n]The melting points of the enantiomeric forms of the sulfate are much higher than those in the literature (213, 210°)[15], and the analyses for the specimens of the methiodides were higher than the acceptable limits. This is due to contamination of the tertiary bases by tropine, with some tropine methiodide occurring in the enantiomeric forms of the methiodide, but this should have little effect on the biological results. [o]Molar rotations at *c* 1 × 10^{-2} *M* (water).

the responses to the alternate high and low concentrations of carbachol in the presence of the antagonist roughly matched the control responses produced initially. With some compounds the responses to the increased concentrations of carbachol in the presence of the antagonist became regular within a few minutes but with more potent compounds tested in very dilute solution it was necessary to wait 20 min or even longer. The regularity of the responses was taken to indicate that the antagonist had come into equilibrium with the receptors. The dose ratio produced by the particular concentrations of antagonist was calculated by comparing the concentrations of carbachol used in the presence and in the absence of the antagonist and taking into account also the actual size of the responses.[19] The affinity constant was calculated from the dose ratio and the concentration of the antagonist according to the Gaddum-Schild equation.[21] It was assumed the estimates were log normally distributed[1,20] and the mean value of log *K* was calculated ± the standard error usually

based on at least five estimates, each obtained with a separate piece of ileum.

Every antagonist was tested in at least two concentrations in order to check that it was acting competitively and usually the concentrations were chosen so that the dose ratios lay between 10 and 1000. Results already obtained[7] have shown that the actions of the enantiomeric forms of benzhexol and of cyclohexylphenylglycoloylcholine, and of mixtures of these enantiomers, are consistent with competitive antagonism and the related compounds studied in this work appear to behave similarly. Values obtained with racemic forms of many of the compounds also indicated that they were acting competitively (see Results). The difference between the estimates of log K for two enantiomers is the log of the stereospecific index (log SSI).

Results

Stereochemical Purity. The esters in any one series of enantiomers were all prepared from the same sample of resolved acid and should therefore all have the same stereochemical purity unless racemization occurred during esterification or the subsequent quaternization. Esters of tropic acid, including hyoscyamine, are relatively rapidly racemized by alkali (though the acid itself is not)[22] so the derivatives of hyoscine and hyoscine in part E of Table I may be partly racemized. It was found, however, that the quaternary salts could be refluxed in acetonitrile for hours without any significant change in rotation, so the metho and etho compounds, which are formed very rapidly, should have much the same stereochemical purity as the base from which they were made.

The racemization is thought to involve an enolic intermediate[22,23] and may therefore occur with esters of mandelic acid and cyclohexylphenylacetic acid. Although synthetic methods involving alkali, such as transesterification, were avoided, it was found that racemization also occurred to some extent in other situations. Cyclohexylphenylacetyl chloride racemized detectably when distilled, for instance, and the preparation of homatropine which involved heating with hydrogen chloride for some time has clearly not produced satisfactory derivatives. In the milder conditions used to make the other mandelic esters and the cyclohexylphenylacetic esters there is some evidence from the biological results (see below) that racemization has not occurred to any appreciable extent, however, and it should not have occurred at all in the synthesis of the derivatives of cyclohexylphenylglycolic and α-methyltropic acids, which lack an enolizable hydrogen atom. From the molar rotations in Table I the two enantiomeric forms in most series appear to be of roughly equal stereochemical purity. The error in the estimate of the rotations is between 1 and 2%[7] but is greater when the angle measured is less than 100 mdeg and so readings at 300 nm are more reliable than those at 589 nm.

Estimates of Log K. Estimates of log K and the values of log SSI derived from them are shown in Table I. The values of log K for the enantiomeric forms of cyclohexylphenylglycoloylcholine and for the tertiary dimethylamino compound agree well with those obtained by Brimblecombe, *et al.*,[5] whose results give values of log SSI for the trimethylammonium compound of 2.28 (*cf.* 2.39) and for the dimethylamino compound of 1.99 (*cf.* 1.89). For the trimethylammonium compound prepared by Ellenbroek, *et al.*,[4] tested on rat intestine, the stereospecifity was 100 (log SSI 2.0) and for the corresponding α-methyltropic ester of choline the stereospecificity was 300 (log SSI, 2.48; *cf.* 2.06 in this work with guinea-pig ileum).

The results for hyoscyamine (log SSI 2.52) indicate slightly greater stereospecificity than was observed by Long, *et al.*,[24] who obtained a stereospecific index of 250 (log SSI, 2.40) in experiments on rabbit intestine.

Table II. Values of Log SSI for the Mandelic Esters (A), Cyclohexylphenylacetic Esters (B), Cyclohexylphenylglycolic Esters (C), and α-Methyltropic Esters (D)[a] and for Derivatives of Hyoscyamine (E), Hyoscine (F), and Homatropine (G)

	A	B	C	D
$^{+}NMe_2H$		0.6	1.9	1.8
$^{+}NMe_3$		0.5	2.4	2.1
$HN^{+}(CH_2)_3CH_2$		0.4	1.7	2.5
$^{+}NEt_2H$		0.2	1.5	1.9
$^{+}NMe_2Et$		0.5	2.2	2.2
$MeN^{+}(CH_2)_3CH_2$	0.4	0.4	2.1	1.7
$HN^{+}(CH_2)_4CH_2$		−0.3	2.1	2.1
$^{+}NEt_2Me$	0.1	0.4	1.9	1.8
$MeN^{+}(CH_2)_4CH_2$	0.4	0.0	2.1	1.7
$EtN^{+}(CH_2)_3CH_2$	0.5	0.4	2.0	1.6
$^{+}NEt_3$	0.0	0.1	1.6	1.7
$EtN^{+}(CH_2)_4CH_2$	0.5	−0.1	1.8	2.3
Range	0.5	0.9	0.9	0.9
	E	F	G	
Base	2.5	1.8	0.5	
MeI	1.9	1.1	0.6	
EtI	1.7	1.5	0.2	
Range	0.8	0.7	0.4	

[a] The onium groups are arranged roughly in increasing order of size.

Errors. From experiments in which the same compound has been tested at different times and by different people,[1,7,25] it seems that in this type of experiment the statistical assessments of the errors within one group of measurements are an underestimate of the real errors. Values of log K which differ by less than 0.1 log units should probably not be considered to be different from each other. Because the stereospecific index is the ratio of two affinity constants, it follows that values of log stereospecific index which differ by less than 0.2 log units should probably not be considered to be different from each other. A check on the errors and on the competitive nature of the actions of the compounds can be made by using the affinity constants for the individual enantiomers to calculate the value expected for the racemate[7] and comparing these values with estimates obtained with the racemate in separate experiments. These values are included in Table I. For 18 pairs of enantiomers the discrepancy between the predicted and observed values was less than 0.1 log units, for seven pairs it was between 0.1 and 0.2 log units, and in only one instance was it bigger than this (0.26 for atropine ethiodide; there are chemical reasons for this, discussed in the following paper[26]).

Variation of Stereospecificity within Series. The values of log SSI for the various series are shown in Table II in which the various onium groups are arranged roughly in order of increasing size. For enantiomeric forms of compounds which are competitive antagonists, the stereospecific index can be used to set a lower limit to the stereochemical purity.[7] In the cyclohexylphenylglycolic and α-methyltropic esters, where racemization should not have occurred, the highest values of the stereospecific index can therefore be used to set a lower limit to the stereochemical purity of each series, which is 99.59 and 99.70%, respectively (corresponding to values of log SSI = 2.39 and 2.52). Even allowing for the estimated errors in log SSI, the stereochemical purity in these series should be better than 99.36 and 99.52%, respectively, and within these series the values of log SSI differ by up to 0.9 log units.

The cyclohexylphenylacetic esters are much less stereospecific and the highest value of log SSI (0.6) only indicates that the stereochemical purity should be better than 78%. If the values of log SSI are low because the compounds have racemized during synthesis, differences between the values should also be low but, in fact, the range of values of log SSI in this series is comparable with that observed in the series whose stereochemical purity is known to be high. The results therefore suggest that the cyclohexylphenylacetic esters did not racemize appreciably during their synthesis. With the mandelic esters also there is low stereospecificity but it is difficult to judge the extent to which racemization may have occurred from the range of values of log SSI because the series is incomplete. The results obtained with the compounds in the lower part of Table II confirm that there can be appreciable differences between the stereospecificity of closely related compounds. Methylation of hyoscyamine, for instance, reduces the stereospecificity fourfold.

In general, an increase in the size of the onium group causes a decrease in stereospecificity (Figure 1), though there are exceptions. The change from methylpiperidinium to ethylpiperidinium, for example, reduces log SSI from 2.1 to 1.8 in the cyclohexylphenylglycolic esters but increases it from 1.7 to 2.3 in the α-methyltropic esters and from 0.3 to 0.5 in the mandelic esters. There is some similarity between the results with the mandelic and α-methyltropic esters which distinguishes them from the results obtained with the other esters and possibly this is due to the presence of one large (benzene) ring in the former compared with two large rings (benzene and cyclohexyl) in the latter. There is no evidence to support Pfeiffer's rule[27] which would require high stereospecificity to be associated with high affinity.

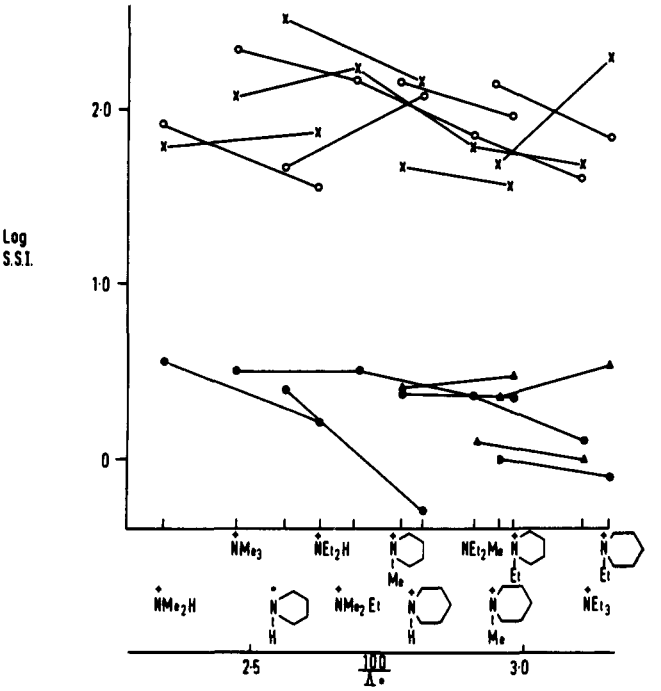


Figure 1. Values of log stereospecific index (log SSI) plotted against size of the onium group; the quaternary groups are arranged in order of size assessed from values of $100/\Lambda_0$ based on conductance measurements with ethyl quaternary salts [ref 1 and B. M. Lowe and H. M. Rendall, *Trans. Faraday Soc.*, 67, 2318 (1971)]. The tertiary compounds have been assigned values arbitrarily: •, cyclohexylacetic esters; ○, cyclohexylglycolic esters; ▲, mandelic esters; X, α-methyltropic esters.

Table III. Effects on Log K for Acetylcholine Receptors of the Guinea-Pig Ileum of Altering the Onium Group in Compounds of the Type $-CH_2CH_2N\lessgtr$ from $^+NMe_3$[a]

	Maximum increase	Minimum increase	No. of comparisons
$^+NMe_2H$	−0.280	−0.781	6
$HN^+(CH_2)_3CH_2$	−0.066	−0.932	6
$^+NEt_2H$	0.499	−0.358	6
$^+NMe_2Et$	0.722	0.036	18
$MeN^+(CH_2)_3CH_2$	0.551	−0.227	18
$HN^+(CH_2)_4CH_2$	0.327	−0.746	6
$^+NEt_2Me$	0.893	0.076	18
$MeN^+(CH_2)_4CH_2$	1.082	−0.477	18
$EtN^+(CH_2)_3CH_2$	1.035	−0.157	18
$^+NEt_3$	1.252	−0.303	18
$EtN^+(CH_2)_4CH_2$	0.992	−0.499	18

[a] The figures are based on the results of Abramson, *et al.*,[1] as well as on the present work. The corresponding increments in the free energy of adsorption (in kcal/mol) can be obtained by multiplying by 1.42.[20] The onium groups are arranged in increasing order of size.

Discussion

A change in structure, such as an increase in size, can increase affinity because it provides additional points for attachment to the receptor. This, however, may disturb the binding of the rest of the molecule at the receptor and it may also affect binding by altering the preferred conformation of the drug in solution or by altering the distribution of electrons within the molecule. The effect on binding is therefore the net result of the positive contribution which the group makes, offset by the disturbance it causes to existing binding, and the effects due to changes in preferred conformation and electron distribution, which may be positive or negative.

In molecules of the type considered here it has been suggested that substituents can produce very large disturbances in the binding of the rest of the molecule at the receptor.[1] The replacement of hydrogen by phenyl in compounds such as pentyltrimethylammonium or phenylpentyltrimethylammonium, for instance, increases log K by 1.2–1.3, indicating an increase in the free energy of adsorption of about 1.8 kcal (7.5 kJ)/mol. It has been calculated, however, that the additional contribution due to the phenyl group is actually 4.3 kcal/mol but that the presence of this group reduces the binding of the rest of the molecule by 2.5 kcal/mol. This does not take into consideration the possible effects of the phenyl substituent on affinity by altering the preferred conformation or the electron distribution. In the particular compounds studied these effects were not known. In some situations they could be large but in series of enantiomeric pairs they must be the same for each isomer.

The changes in log SSI produced by altering the onium group in the series of compounds studied in the present work therefore indicate differences between the positive effects of the alteration on the binding of the two enantiomers and/or differences between the effects of the alteration on the disturbance in the binding of the rest of the molecule. Although the positive effects on affinity of a change in structure cannot be measured, it should be possible to obtain some idea of their upper limit by observing the maximum effect of the change in structure on the affinities of a large number of compounds. This maximum

increase might correspond to the situation in which the introduction of the substituent allows it to interact maximally with the most suitable group in the receptor without disturbing the binding of the rest of the molecule.

The maximum effects on affinity of changes in the composition of the onium group are shown in Table III, which includes results obtained in earlier work.[1] Although these may be underestimates because only a limited number of compounds have been examined, they are not much bigger, and in some instances apparently smaller, than the variations observed in the values of log SSI (0.9 log units). It seems unlikely, then, that the variation in stereospecificity arises simply because the positive contribution to binding is bigger in one enantiomer than another. It appears likely that the change in composition affects the stereospecificity because it disturbs the binding more in one enantiomer than the other, though there may be differences in the positive contribution as well.

Acknowledgments. We thank Miss Margaret Harrison for her highly competent and enthusiastic technical assistance, Dr. R. R. Ison for valuable discussion, the Faculty of Medicine for the award of a scholarship (to J. D. M. P.), and the Medical Research Council for financial support. We wish particularly to thank Professor G. Maffii, Professor G. Nathanson, and Dr. R. P. Paton for their very generous gifts of resolved material.

References

(1) F. B. Abramson, R. B. Barlow, M. G. Mustafa, and R. P. Stephenson, *Brit. J. Pharmacol.*, 37, 207 (1969).
(2) W. M. Duffin and A. F. Green, *ibid.*, 10, 383 (1955).
(3) V. Scarselli, G. Cignarella, and G. Maffii, *J. Med. Chem.*, 7, 237 (1964).
(4) B. W. J. Ellenbroek, R. J. F. Nivard, J. M. van Rossum, and E. J. Ariëns, *J. Pharm. Pharmacol.*, 17, 393 (1965).
(5) R. W. Brimblecombe, D. M. Green, T. D. Inch, and P. B. J. Thompson, *ibid.*, 23, 745 (1971).
(6) R. B. Barlow, *ibid.*, 23, 90 (1971).
(7) R. B. Barlow, F. M. Franks, and J. D. M. Pearson, *ibid.*, 24, 753 (1972).
(8) H. Thies and F. W. Reuther, *Naturwissenschaften*, 41, 230 (1954).
(9) E. M. Hancock and A. C. Cope, *Org. Syn.*, 25, 25 (1945).
(10) K. Hoffman and H. Schellenberg, *Helv. Chim. Acta*, 30, 292 (1947).
(11) C. H. Boehringer Sohn, British Patent 708,370 (1954); *Chem. Abstr.*, 49, 11031 (1955).
(12) B. W. J. Ellenbroek, Ph.D. Thesis, Catholic University of Nijmegen, Rotterdam, Bronder Offset, 1964.
(13) T. D. Inch, R. V. Ley, and P. Rich, *J. Chem. Soc. C*, 1693 (1968).
(14) A. Vecchi and G. Melone, *J. Org. Chem.*, 24, 109 (1959).
(15) G. Werner and K. Miltenberger, *Justus Liebigs Ann. Chem.*, 631, 163 (1960).
(16) K. Miescher and K. Hoffman, *Helv. Chim. Acta*, 24, 458 (1941).
(17) R. R. Burtner and J. W. Cusic, *J. Amer. Chem. Soc.*, 65, 262 (1943).
(18) H. A. D. Jowett and F. L. Pyman, *J. Chem. Soc.*, 1020 (1909).
(19) Edinburgh Staff, "Pharmacological Experiments on Isolated Preparations," 2nd ed, E. & S. Livingstone, Edinburgh, 1970, p 73.
(20) R. B. Barlow, K. A. Scott, and R. P. Stephenson, *Brit. J. Pharmacol.*, 21, 509 (1963).
(21) H. O. Schild, *ibid.*, 4, 277 (1949).
(22) J. Gadamer, *J. Prakt. Chem.*, 87, 312 (1913).
(23) P. F. Frankland, *J. Chem. Soc.*, 712 (1913).
(24) J. P. Long, F. P. Luduena, B. F. Tullar, and A. M. Lands, *J. Pharmacol. Exp. Ther.*, 117, 29 (1956).
(25) R. B. Barlow, F. M. Franks, and J. D. M. Pearson, *Brit. J. Pharmacol.*, 46, 300 (1972).
(26) R. B. Barlow, M. Harrison, R. R. Ison, and J. D. M. Pearson, *J. Med. Chem.*, 16, 564 (1973).
(27) C. C. Pfeiffer, *Science*, 124, 29 (1956).

Linear Free Energy Relationships in the Hydrolysis of Some Inhibitors of Acetylcholinesterase†

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The spontaneous hydrolysis of a series of diethyl phosphates I and 1,3,2-dioxaphosphorinane 2-oxides II has been studied in a number of buffers. The present study shows that the hydroxide ion catalysis of both series I and II obeys a linear Brönsted relationship over a pK_a range extending from $pK_a = 10$ to -7. On the other hand, the data for the water reactions were less conclusive. The possibility that the chloro derivatives hydrolyze *via* an SNl (phosphorylium ion) mechanism has been excluded. The sharp break in the Brönsted plots for the second-order rate constants in the reactions of series I with eel cholinesterase which was previously reported by us is, therefore, believed to result from a special property of the enzyme reaction. We have rationalized the enzyme reaction in terms of a mechanism involving the formation of two reversible intermediates along the reaction coordinate.

The inhibition of cholinesterase by organophosphates is a nucleophilic substitution reaction in which the enzyme serves as a nucleophile. Recently, we have shown that a plot of the logarithm of the bimolecular rate constant for the inhibition of eel ChE by diethyl phosphates I *vs.* the pK_a of the leaving group has a sharp break in the Brönsted plot.[1] Very limited data for 1,3,2-dioxaphosphorinane 2-oxides II are consistent with a break in the curve which is much less sharp.[2] The observed nonlinearity suggests a change in the rate-determining step of a multistep reaction. However, in order to determine whether this phenomenon that we observed in the enzymatic reaction with I and II is specific to the enzyme, a kinetic study of the hydrolysis of these inhibitors in the presence of other nucleophiles is required. The data presented here provide some information on the linearity of Brönsted plots for the hydrolysis of compounds I and II (k_{OH^-}, k_{H_2O}), by extending the pK_a range to -7

I II

X = Cl, F, *p*-nitrophenoxy

†This work was supported by Grant GB 24193, National Science Foundation, and Grants NS 07156 and NS 09197, National Institutes of Health.