Structure–Activity Relations. I. A Series of Antagonists of Acetylcholine and Histamine at the Postganglionic Receptors

KEITH BOWDEN AND R. C. YOUNG

Department of Chemistry, The University of Essex, Colchester, Essex, England

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A series of aminoester hydrochlorides, which are antagonists of acetylcholine and histamine at postganglionic receptors, have been synthesized. Their activity has been successfully correlated by Hansch linear free energy relations involving polar, steric, and partition substituent constants. The results are related to receptor–drug interactions.

Hansch and his coworkers[1] have developed linear free energy relations which can correlate biological activity and chemical structure. The relations, 1 and 2, together with simplified versions, have been proposed to relate the molar concentration, C_x, of a substituted compound of a series, which all cause an equivalent biological response, to the hydrophobic

$$\log (1/C_x) = -a\pi^2 + b\pi + \rho\sigma + c \quad (1)$$

$$\log (1/C_x) = b\pi + \rho\sigma + c \quad (2)$$

bonding or partition constant, π, and the Hammett constant, σ. The constants a, b, ρ, and c are obtained from the regression analysis and define the response of the biological system to structural features. The partition constant, π, is defined from a model system with partition between 1-octanol and H_2O.[1,2] The Hammett substituent constants are measures of the polar effects of *meta*- and *para*-substituted benzene derivatives.[3] However, σ_I values could be used to correlate polar effects in aliphatic systems.[4] Steric effects can be estimated in these systems by the steric substituent constant, E_s.[5] These two latter parameters have been used very successfully in the Taft–Ingold equation (3) to correlate reactions which depend on both factors.[5,6]

$$\log (k/k_0) = \rho_I\sigma_I + \delta E_x \quad (3)$$

Recently, Cammarata[7] has shown that the electronic polarizability, P_e, can be used in correlating biological activities. This parameter could relate either the charge transfer ability or spatial demands of the substituent.

Many studies have been made of the structure–activity relations for antagonists of acetylcholine at the postganglionic receptors.[8] These drugs interfere with the transmission of nerve impulses mediated by acetylcholine at the junctions of the postganglionic cholinergic nerves. The best-known antagonist at these sites of the "muscarine-like" actions of acetylcholine is atropine. A vast number of esters of aminoalcohols have been studied and their activity usually is measured by their ability to antagonize contractions in isolated guinea-pig ileum. Histamine can also stimulate the ileum. It probably has a site of action distinct from that of acetylcholine, but similarities exist.[9] Many antagonists of acetylcholine also inhibit the action of histamine but to a different degree.

In the present study, a series of aminoester hydrochlorides have been synthesized and their antagonism to acetylcholine and histamine has been measured. These activities have been correlated using structural parameters and the results related to drug–receptor interactions.

Experimental Section[10]

Acids, Acid Chlorides, 2-Dialkylaminoethanols, and 2-Dialkylaminoethyl Chlorides.—These compounds were prepared by known methods[11–13] and, after purification, had physical constants in good agreement with literature values, except for the acid described below.

2-Chlorofluorene-9-carboxylic Acid.—This acid was prepared by metalation of 2-chlorofluorene with ethereal *n*-BuLi, followed by treatment with solid CO_2.[12a] After recrystallization from MeOH–H_2O, the colorless acid had mp 213–215°. *Anal.* ($C_{14}H_{11}ClO_2$) C, H, Cl, O.

Ester Hydrochlorides.—These were prepared by reacting either the acid chloride with the dialkylaminoalcohol in C_6H_6 or the acid with the dialkylaminoethyl chloride in *i*-PrOH or PhMe.[11] An exception to this is 2-diethylaminoethyl 2-aminofluorene-9-carboxylate·HCl, which was prepared by direct reduction of the 2-nitro ester hydrochloride.[11] The ester hydrochlorides were purified by recrystallization, sometimes after conversion into base and treatment with ethereal HCl (see Table I).

pK_a Values of the Ester Hydrochlorides.—These were determined in 50% v/v EtOH–H_2O, at 25 (±0.1)°, at 0.004 *M* concentration using a method described previously.[14] Those fluorene esters having an unsubstituted 9-position have a second pK_a due to formation of the carbanion at higher pH (pK_a of diethylaminoethyl fluorene-9-carboxylate is approximately 12 in H_2O). The likelihood of interference from this second ionization is considerably reduced in 50% EtOH–H_2O due to the differential effect

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(9) (a) Reference 8b Chapter X; (b) D. J. Triggle, "Chemical Aspects of the Autonomic Nervous System," Academic Press Ltd., London, 1965, p 120.

(10) Melting points were determined on a Kofler hot-stage microscope and are corrected. Where analyses are indicated by symbols of the elements, analytical results were within ±0.4% of the theoretical values.

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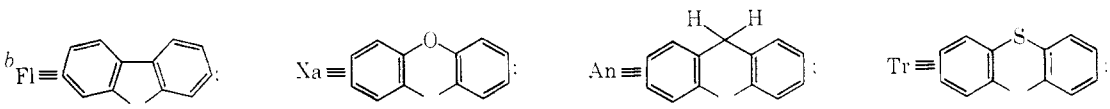
TABLE I

ANTAGONIST ACTIVITY, pK_a VALUES, AND PHYSICAL PROPERTIES OF AMINOESTER HYDROCHLORIDES

	Aminoester hydrochloride $RCO_2CH_2CH_2NEt_2HCl$, R	Antagonism[a] (−log (1/C_{50})) Acetylcholine, log (1/C_{50})	Antagonism[a] (−log (1/C_{50})) Histamine, log (1/C_{50})	pK_a in 50% ethanol–water at 25°	Mp, °C Found	Mp, °C Lit.	Formula	Anal.
1	$PhCH_2$	4.43_4	4.00_3	8.06	*d*	bp 125		
2	Ph_2CH	5.80_2	6.54_1	7.92	111–112	112		
3	Ph_2CMe	6.72_3	5.91_4	7.86	161–162	161–162		
4	$Ph_2CCH_2CH{=}CH_2$	5.71_4	5.66_4	7.76	106–107	108.5–110		
5	XaCH	6.91_4	6.60_4	7.79	157–158	159–160		
6	TxCH	5.87_5	6.96_5	7.97	193–195	195		
7	AnCH	6.15_3	7.30_1	7.85	168–169	170–171		
8	FlCH	6.55_3	5.97_1	7.86	141–142	143–144		
9	FlCMe	6.96_5	6.79_3	7.95	154–155	118–120[e,f]	$C_{21}H_{26}ClNO_2$	C, H, Cl, N, O
10	$FlCCH_2Me$	5.49_4	6.42_6	7.94	171–172	168–169		
11	$FlCCHMe_2$	5.41_2	6.29_7	7.86	202–203	*f*	$C_{23}H_{30}ClNO_2$	C, H, Cl, N, O
12	$FlC(CH_2)_3Me$	5.66_6	5.91_4	7.98	133–134	*g*	$C_{24}H_{32}ClNO_2$	C, H, Cl, N, O
13	$FlCCMe_3$	5.12_5	5.92_3	7.88	194–195	*f*	$C_{24}H_{32}ClNO_2$	C, H, Cl, N, O
14	$FlCCH_2Ph$	4.75_1	4.80_7	7.83	116–118	*f*	$C_{27}H_{30}ClNO_2$	C, H, Cl, N, O
15	FlCOH	6.84_2	6.19_7	7.90	198	204		
16	2-Br(FlCH)	5.69_1	5.62_3	7.75	152.5–153.5	*f*	$C_{20}H_{23}BrClNO_2$	C, H, Br, Cl, N, O
17	2-Cl(FlCH)	6.08_7	5.90_8	7.78	149.5–150.5	*f*	$C_{20}H_{23}Cl_2NO_2$	C, H, Cl, N, O
18	2-OH(FlCH)	5.74_2	5.21_6	7.83	210–212	*f*	$C_{20}H_{24}ClNO_3$	C, H, Cl, N, O
19	2-OMe(FlCH)	4.76_2	5.37_1	7.81	73–75	*i*	$C_{21}H_{26}ClNO_3$	C, H, Cl, N, O
20	$2\text{-}NO_2$(FlCH)	5.16_3	5.30_2		120–121	*j*	$C_{20}H_{23}ClN_2O_4$	C, H, Cl, N, O
21	$2\text{-}NH_2$(FlCH)	5.25_9	5.01_7	7.85	92–93	92–94		
22	$FlCH(NMe_2)_1$	5.29_6	5.65_3	7.46	143	*k*	$C_{19}H_{29}ClNO_2$	C, H, Cl, N, O
23	$FlCH(NBu_2)$	5.70_1	5.04_8	7.11	154	165		
24	FlCH(Trop)	7.39_2	7.06_3	8.74	204–205	200–201		
25	$FlCH(PropNEt_2)_1$	4.95_8	5.02_4		141–142	143–144		

[a] C_{50} values (*M*) are normally reproducible to less than ±8% and are the average of, at least, two separate determinations. Atropine sulfate has a value of log (1/C_{50}) equal to 7.63_4 for antagonism to acetylcholine under the same conditions. pK_a values are the average of, at least, two determinations and are reproducible to ±0.03 unit.



NMe_3 ≡ $2\text{-}Me_2N(CH_2)_2$ ester; NBu_2 ≡ 2-*n*-Bu_2N ester; Trop ≡ tropan-3α-yl ester; $PropNEt_2$ ≡ 3-dimethylaminopropyl ester. [c] References 11 and 16. [d] Wax-like solid crystallized from oil after desiccating and had an indistinct melting point. Analysis results confirmed purity. [e] Poor agreement between literature and found melting point could not be reconciled. Our analytical results confirmed purity. [f] Colorless needles from *i*-PrOH. [g] Colorless plates from *i*-PrOH. [h] Colorless plates from *i*-PrOH. [i] Pale yellow needles from PhMe. [j] Pale yellow needles from EtOAc–*i*-PrOH. [k] Pale yellow needles from *i*-PrOH–Et_2O.

of the solvent change on the ionizations. The pK_a values of the ester hydrochlorides in this medium will be close to those in H_2O.[15] The 2-nitro ester gave no characteristic inflection point in the titration and a green coloration of the solution with blue fluorescence could be observed, indicating formation of the carbanion. The values found are shown in Table I.

Pharmacology.—The antagonist activity to acetylcholine (ACh) was assessed on the isolated guinea pig ileum. The ileum was suspended in oxygenated Tyrode's solution at 38 (±0.5)°. After regular responses had been obtained, the antagonist was added at least 2 min prior to stimulation with ACh·Br (0.442 *M*). The contractions were elicited at regular intervals of, normally, 5 min. Each compound was tested in, at least, four concentrations on segments of terminal ileum of, at least, two guinea pigs. The activity, C_{50}, was obtained as the dose required to produce a 50% inhibition of spasm induced by ACh by interpolation. The assessment of the antagonism to histamine was conducted in a similar manner, using the ileum suspended in Tyrode's solution containing histamine·2HCl. In Table I are shown the log (1/C_{50}) values. For those compounds that had been previously tested,[9,13,16] the relative values obtained compared closely, in almost every case, with those in this study. All the pharmacological results were obtained by Professor J. J. Bein, Dr. R. Jacques, and their coworkers at Ciba Ltd., Basle, Switzerland, to whom the authors are extremely grateful.

Calculations. Substituent Constants.—The polar substituent constants were either obtained from the literature (σ_m)[17] or from our previously published study (σ_I).[6] The steric substituent constants, E_s, required were also obtained from the latter study.[7] The electronic polarizabilities, P_e, were calculated from literature data.[18] All these values are shown in Tables II and III, together with the other substituent constants whose derivation is detailed below.

The Steric Substituent Constant, *R*.—These have been calculated as the distance in ångströms from the aromatic carbon atom to which a substituent is bonded to the periphery of the van der Waals' radius of the substituent, relative to that of H, using known bond distances[19] and van der Waals' radii.[20] Charton[21] has recently used a similar procedure in evaluating steric substituent constants.

Partition Constants.—The partition constants, π, have been calculated by direct measurements of partition between H_2O and 1-octanol, together with the additivity principle of Hansch.[2] Partition coefficients, P_x, were measured for the parent acids, with H_2O:1-octanol ratios of 20 and 40, using a spectrophoto-

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TABLE II

SUBSTITUENT CONSTANTS[a]

	Substituent[b]	σ_1	E_s	π
1	$PhCH_2$	0.11$_5$	−0.37	2.22
2	Ph_2CH	0.19	−1.46	2.98
3	Ph_2CMe	0.15	−3.55	3.50
4	$Ph_2CCH_2CH{=}CH_2$	0.17$_5$	−4.33	4.75
5	XaCH	0.31$_5$	−1.29	3.04
6	TxCH	0.25$_5$	−1.75	3.38
7	AnCH	0.23$_5$	−1.29	3.59
8	FlCH	0.22$_5$	−1.10	3.61
9	FlCMe	0.15$_5$	−1.73	4.13
10	$FlCCH_2Me$	0.13$_5$	−1.97	4.61
11	$FlCCHMe_2$	0.12$_5$	−3.30	5.04
12	$FlC(CH_2)_3Me$	0.14$_5$	−2.09	5.69
13	$FlCCMe_3$	0.08$_5$	−4.12	5.63
14	$FlCCH_2Ph$	0.23	−2.16	6.31
15	FlCOH	0.47	−0.90	1.81
16	2-Br(FlCH)	0.31		4.60
17	2-Cl(FlCH)	0.31		4.44
18	2-OH(FlCH)	0.23$_5$		3.23
19	2-OMe(FlCH)	0.26		3.75
20	$2\text{-}NO_2$(FlCH)	0.40$_5$		3.56
21	$2\text{-}NH_2$(FlCH)	0.20$_5$		2.36

[a] σ_1 constants are literature values or calculated from literature data;[6] E_s constants are literature values;[6] and π constants are from this study (see Experimental Section). [b] See Table I.

TABLE III

SUBSTITUENT CONSTANTS[a] (NUCLEAR SUBSTITUTION)

Substituent	σ_m	R	log P_e
H	0.0	0.0	0.23
Br	0.391	1.52	0.97
Cl	0.373	1.22	0.81
OH	0.121	1.24	0.46
OMe	0.115	2.03	0.89
NO_2	0.710	1.81	0.92
NH_2	0.040	1.36	0.56

[a] σ_m constants are literature values;[17] P_e (ml) values (polarizability) were calculated from the literature data;[18] and R constants are from this study (see Experimental Section).

Results and Discussion

Antagonism to ACh.—Our first attempts to correlate the antagonist activity of the amino ester hydrochlorides involved the complete series of diethylaminoethyl ester hydrochlorides, $RCO_2CH_2CH_2NEt_2 \cdot HCl$ (n = 21, Table I, **1** to **21**). All these salts have pK_a values which are closely related[23] (mean value is 7.87 ± 0.06) due to the lack of proximity of the substituent to the NEt_2 group. Thus, the state of ionization of these aminoesters in any test medium is almost the same and nearly independent of the nature of the group R. Attempts have been made to correlate the antagonist activity with the polar and partition constants in Hansch-type relations in Table IV. The single parameter correlations have been omitted from this and later tables as no useful or successful relations were observed. Unfortunately none of the attempted correlations (1–3) shown has a high enough Student's t test or correlation coefficient, *i.e.*, $r < 0.7$, to warrant discussion. Steric parameters cannot be usefully applied, as E_s values for the 2-substituted fluorene esters will be almost the same as the unsubstituted fluorene ester.

TABLE IV

CORRELATIONS OF ANTAGONISM OF ACETYLCHOLINE BY ALL THE 2-DIETHYLAMINOETHYL ESTERS (n = 21)[a]

					r	s
1	$-0.218\pi^2(1.29)$	$+0.871\pi(1.07)$		$+4.495$	0.387	0.714
2		$-0.114\pi(0.75)$	$+1.615\sigma_1(0.88)$	$+5.851$	0.331	0.730
3	$-0.136\pi^2(1.37)$	$+0.996\pi(1.21)$	$+1.812\sigma_1(1.01)$	$+3.733$	0.445	0.714

[a] n = number of compounds; r = correlation coefficient; s = standard deviation and the quantity in parentheses is the Student's t test (lit. G. W. Snedecor, "Statistical Methods," 5th ed, Iowa State University Press, Ames, Iowa, 1956) for the significance of the regression variable above.

TABLE V

CORRELATIONS OF ANTAGONISM OF ACETYLCHOLINE BY THE NONNUCLEAR SUBSTITUTED 2-DIETHYLAMINOETHYL ESTERS (n = 15)[a]

						r	s
4	$-0.174\pi^2(1.61)$	$+1.165\pi(1.30)$			$+4.300$	0.571	0.702
5		$-0.123\pi(0.72)$	$+3.471\sigma_1(1.50)$		$+5.695$	0.557	0.711
6		$-0.329\pi(1.64)$		$-0.124E_s(0.57)$	$+6.960$	0.450	0.764
7			$+4.837\sigma_1(2.14)$	$-0.084E_s(0.46)$	$+4.750$	0.541	0.720
8	$-0.317\pi^2(3.73)$	$+2.586\pi(3.51)$	$+6.646\sigma_1(3.68)$		-0.197	0.835	0.491
9	$-0.169\pi^2(1.43)$	$+1.109\pi(1.08)$		$-0.030E_s(0.14)$	$+4.379$	0.572	0.733
10		$-0.217\pi(1.09)$	$+3.955\sigma_1(1.66)$	$-0.198E_s(0.95)$	$+5.562$	0.602	0.714

[a] For footnotes see Table IV.

metric method and were corrected for ionization of the acids.[2a] Acids were 10^{-3} to 10^{-4} M in aqueous phase. The partition constant, π, is defined, in this study, as $\log P_x - \log P_{Me}$ so that π for Me equals 0.0. A value for $PhCH_2$ on this scale can be calculated as 2.22.[2a] The parameters for the other acids were then calculated from their log P_x values relative to that of phenylacetic acid. The π values of the very insoluble carboxylic acids (Table II, substituents 4, 9–14, 16–21) were estimated from values for the substituents calculated from our and from literature data.[1,2,22] The lack of additivity of effects of the phenyl groups (Table II, substituents 1, 2) is considered to arise from mutual shielding of these groups reducing their effective hydrophobicity. The parameters are considered to be certain to ±5%.

Regression Analysis.—Regression analyses were made using multiple regression analysis (least squares).

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(23) The ρ value for the 2-substituted fluorene-9-carboxylic diethylaminoethyl esters (using σ_m) is only 0.24 (pK_a° = 7.86, r = 0.960, n = 6).

It became obvious that a separation of the diethylaminoethyl esters could be made into two series. The first series (n = 15, Table I, **1–15**) includes all those not having nuclear substitution on the periphery of the aromatic group and the second series (n = 7, Table I, **8**, **16–21**) includes those having such nuclear substitution, together with the unsubstituted reference compound of this series.

Attempted correlations of the antagonist activity of the nonnuclear substituted diethylaminoethyl esters with polar, steric, and partition substituent parameters are shown in Table V. Only one correlation has a high statistical significance (Table V, regression 8). This relation, shown below (eq 4), gives a very good account of the effects of substitution. The calculated

$$\log (1/C_{50}) = -0.317\pi^2 + 2.596\pi + 6.646\sigma_1 - 0.197 \quad (4)$$

and observed values of log $(1/C_{50})$ agree to a mean value of ± 0.352. The optimum partition constant[24] of π_0 can be calculated from this regression to be 4.08 for R in $RCO_2CH_2CH_2NEt_2 \cdot HCl$. This parabolic dependence of drug action upon lipophilic character is typical of a large number of different drug systems previously investigated by Hansch,[1,24] when a wide range of lipophilicity is investigated. This appears to indicate the importance of partition out of the aqueous phase and onto the receptor which is associated with hydrophobicity of the group R. A less likely explanation appears to be the occurrence of hydrophobic interactions with the receptor. The dependence of the activity on polar effects, with ρ_I equal to 6.6 and no significant contribution from steric effects, as measured by E_s, appears to be directly related to the drug's ability to effectively interact with the receptor. Any interaction involving spatial demand at the C=O (dependent on E_s) or H bonding *from* the receptor *to* the C=O (facilitation indicated by negative ρ_I value) therefore appears unlikely. A possible occurrence appears to be an electrostatic interaction at the receptor site between the substituent dipole on the antagonist and an anionic site. The substituent constant, σ_I, appears mainly to arise from the electrostatic field of the charged or dipolar substituent.[25] Model calculations[26] using estimates of the molecular parameters, effective dielectric constant, and stereochemical relations in the antagonist–receptor complex indicate this explanation is energetically reasonable. The activities of a number of related diethylaminoethyl esters have been measured[11,13b] and can be related to those in this study. A selection of these, where substituent constants could be estimated with a good degree of reliability, are shown in Table VI. Reasonable agreement exists between the calculated and observed activities, according to the limits of our regression analysis, *except* for esters having a β-OH group. This disagreement may extend to esters having an α-OH group but to a very much less extent. The difference between the calculated and observed values for the β-OH compounds was about +3 log units. This

TABLE VI

ESTIMATION OF ANTAGONISM OF ACETYLCHOLINE OF CERTAIN 2-DIETHYLAMINOETHYL ESTERS[a] ($RCO_2CH_2CH_2NEt_2 \cdot HCl$)

Substituent[b] R	Substituent constants π	Substituent constants σ_I	Antagonism to acetylcholine, log $(1/C_{50})$ Obsd	Antagonism to acetylcholine, log $(1/C_{50})$ Calcd
AdCMe	4.11	0.18	5.88	6.24
BiphCH$_2$	3.30	0.12	5.25	5.67
FlC(cyclobexyl)	6.07	0.11	4.55	4.46
PhCH(CH$_2$)$_2$Me	3.49	0.07	5.66	5.41
HOCH$_2$CHPh	0.92	0.16$_5$	6.49	3.01
HOCHPhCH$_2$	0.92	0.10$_5$	5.71	2.61
Ph$_2$COH	1.18	0.44$_5$	6.68	5.38

[a] Calculated according to regression 8, Table V. Observed values from literature data,[11-13b] relative to compound 8, Table I. Substituent constants estimated from literature data[2,9b,c] and this study. [b] See Table I. Biph = *o*-$C_6H_5C_6H_4$.

is equal to a free energy difference of about 4 kcal mole^{-1}, which agrees closely with the energy of a hydroxyl H bond to an oxygen site.[27] This appears to indicate that the β-OH can H bond to an ionic site[28] in a specific manner not accommodated by the linear free energy regression analysis.[29] Our results are consistent with the present knowledge of receptor sites in these systems.[8,30] The active site appears to have two anionic sites separated by a distance of 7–8 Å.[30] One site interacts with the cationic head, while a second site could accommodate the binding from the ester group. A too highly lipophilic antagonist could be diverted to lipoidal rich sites whereas a too hydrophilic antagonist could tend to remain in the aqueous phase or other polar systems. While the latter dependence could be attributed to variations in the rates of absorption if an insufficient period of exposure of the ileum has occurred, this seems unlikely. The quasi-equilibria model of Hansch[1] for this parabolic dependence seems more reasonable.

Attempted correlations of the antagonist activity of the nuclear substituted diethylaminoethyl esters with substituent parameters are shown in Table VII. Two correlations appear to have high statistical significance (Table VII, regressions 13 and 14). The most successful relation, shown below (eq 5), gives an

$$\log (1/C_{50}) = 0.279\pi - 0.754R + 5.634 \quad (5)$$

excellent account of the nuclear substitution. The calculated and observed values of log $(1/C_{50})$ agree to a mean value of ± 0.089. This correlation appears to indicate the unfavorable effects of spatial demand on the periphery of the antagonist. The other successful correlation involving the electronic polarizability very probably arises from the spatial properties of this factor. The dependence on partition relates to that particular part of the parabolic relation found previously which is covered by this more limited series. A reasonable interpretation of the former behavior appears to be the inhibition of close and successful approach of the antagonist to the receptor existing in a "trough-like" depression, for which there is some

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TABLE VII

CORRELATIONS OF ANTAGONISM OF ACETYLCHLORINE BY THE NUCLEAR SUBSTITUTED 2-DIETHYLAMINOETHYL ESTERS (n = 7)[a]

						r	s
11	$+0.001\pi^2(0.00)$	$+0.226\pi(0.07)$			+4.771	0.289	0.708
12		$+0.387\pi(0.98)$	$-1.019\sigma_m(0.88)$		+4.452	0.481	0.649
13		$+0.279\pi(3.69)$		$-0.754R(10.01)$	+5.634	0.982	0.139
14		$+0.649\pi(3.49)$		$-2.194 \log P_e$ (4.38)	+4.755	0.918	0.294
15			$+0.281\sigma_m(0.59)$	$-0.770R(4.77)$	+6.603	0.926	0.279
16	$+0.012\pi^2(0.11)$	$+0.195\pi(0.25)$		$-0.755R(8.69)$	+5.775	0.982	0.160
17		$+0.300\pi(3.19)$	$-0.147\sigma_m(0.50)$	$-0.739R(8.25)$	+5.572	0.984	0.154

[a] For footnotes see Table IV.

TABLE VIII

CORRELATIONS OF ANTAGONISM OF HISTAMINE BY ALL THE 2-DIETHYLAMINOETHYL ESTERS (n = 21)[a]

					r	s
18	$-0.208\pi^2(2.02)$	$+1.699\pi(2.01)$		+2.703	0.430	0.739
19		$+0.037\pi(0.22)$	$+0.659\sigma_I(0.32)$	+5.595	0.079	0.816
20	$-0.213\pi^2(2.01)$	$+1.766\pi(2.02)$	$+0.966\sigma_I(0.51)$	+2.296	0.444	0.755

[a] For footnotes see Table IV.

TABLE IX

CORRELATION OF ANTAGONISM OF HISTAMINE BY THE NONNUCLEAR SUBSTITUTED 2-DIETHYLAMINOETHYL ESTERS (n = 15)[a]

						r	s
21	$-0.283\pi^2(2.58)$	$+2.236\pi(2.46)$			+2.110	0.605	0.713
22		$+0.017\pi(0.08)$	$+2.390\sigma_I(0.85)$		+5.535	0.265	0.863
23		$-0.116\pi(0.50)$		$-0.070E_s(0.28)$	+6.402	0.144	0.886
24			$+3.053\sigma_I(1.13)$	$-0.117E_s(0.53)$	+5.231	0.310	0.851
25	$-0.411\pi^2(4.47)$	$+3.527\pi(4.43)$	$+6.200\sigma_I(3.19)$		−2.046	0.819	0.537
26	$-0.298\pi^2(2.50)$	$+2.417\pi(2.35)$		$+0.096E_s(0.43)$	+1.857	0.613	0.739
27		$+0.040\pi(0.16)$	$+2.684\sigma_I(0.90)$	$-0.120E_s(0.46)$	+5.454	0.297	0.893

[a] For footnotes see Table IV.

TABLE X

CORRELATIONS OF ANTAGONISM OF HISTAMINE BY THE NUCLEAR SUBSTITUTED 2-DIETHYLAMINOETHYL ESTERS (n = 7)[a]

						r	s
28	$-0.068\pi^2(0.33)$	$+0.821\pi(0.57)$			+3.424	0.732	0.298
29		$+0.423\pi(2.63)$	$+0.521\sigma_m(1.10)$		+4.073	0.796	0.265
30		$+0.361\pi(4.28)$		$-0.281R(3.35)$	+4.557	0.935	0.155
31		$+0.508\pi(4.86)$		$-0.861 \log P_e$ (3.06)	+4.229	0.926	0.165
32			$+0.344\sigma_m(0.57)$	$-0.300R(1.47)$	+5.815	0.594	0.352
33	$0.064\pi^2(0.55)$	$+0.810\pi(0.98)$		$-0.281R(3.03)$	+3.799	0.941	0.171
34		$+0.393\pi(3.85)$	$-0.217\sigma_m(0.68)$	$-0.258R(2.66)$	+4.464	0.944	0.167

[a] For footnotes see Table IV.

evidence.[31] This probably indicates one of the reasons for the scatter in the previous series (Table V, regression 8), where it was not possible to allow for the different peripheral spatial demands of the compounds.

The fluorene-9-carboxylate aminoesters studied having variation in the side chain (Table I, **8, 22–25**) are not sufficient to enable an attempt at a quantitative separation. This would be more difficult, in any case, for those tertiary amines whose pK_a values vary widely (7.11–8.74).

Antagonism to Histamine.—The analysis of the antagonist activity of the aminoester hydrochlorides is shown in Tables VIII–X. A very similar pattern of behavior as that noted for antagonism to ACh is observed. No statistically significant correlation of all the esters occurs (Table VIII). The successful correlation of the nonnuclear substituted diethylaminoethyl esters is shown below (eq 6) (Table IX, regression 25).

$$\log (1/C_{50}) = -0.411\pi^2 + 3.527\pi + 6.200\sigma_I - 2.046 \quad (6)$$

The calculated and observed values of log $(1/C_{50})$ agree to a mean value of ±0.406. This correlation is less well-defined than that for the antagonism toward acetylcholine. However, the optimum partition constant of π_0 for this series is 4.29, which compares closely with that found for the previous series. If the receptors for both actions occur in the same or very closely related environment, as is suspected,[9b] the optimum partition factors would be expected to be very similar. The dependence on polar factors also closely compares with the previous analysis. The nuclear substituted series also give a satisfactory correlation with partition and steric factors, as shown below (eq 7). The calculated and observed values

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$$\log (1/C_{50}) = 0.361\pi - 0.281R + 4.557 \quad (7)$$

of $\log (1/C_{50})$ agree to a mean value of ± 0.102. Again the relation corresponds closely to our analysis of antagonism toward acetylcholine. A better study of antagonism toward histamine could be made with more satisfactory and specific antagonists.

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Antimalarials. 8-Chloro-4-(2′-*N*,*N*-dibutylamino-1′-hydroxyethyl)benzo[*h*]-1,6-naphthyridine[1a]

KARL A. ROSEMAN,[1b] MARIBETH M. GOULD, WARNER M. LINFIELD, AND BEN E. EDWARDS

Organic Chemistry Section, IIT Research Institute, Chicago, Illinois 60616

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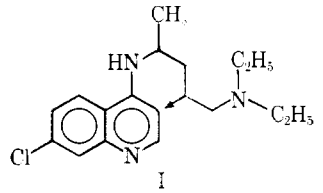
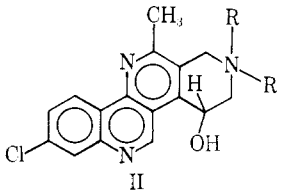
8-Chloro-4-(2′-*N*,*N*-dibutylamino-1′-hydroxyethyl)benzo[*h*]-1,6-naphthyridine (**17**) was synthesized from 4-amino-7-chloroquinoline. While a number of electrophiles failed to react with the weakly nucleophilic 4-amino function of the 4-aminoquinoline, utilization of diethyl ethoxymethylenemalonate, a relatively strong electrophile, provided a route to the tricyclic heteroaromatic compound. Whereas eight of its intermediates lacked antimalarial activity, 8-chloro-4-(2′-*N*,*N*-dibutylamino-1′-hydroxyethyl)benzo[*h*]-1,6-naphthyridine was active against *Plasmodium berghei* in mice. This compound, however, possessed only slight activity against *P. gallinaceum* in chicks and was ineffective against this organism in mosquitoes.

Initially, it was our desire to prepare 2-methyl-4-(*N*,*N*-dialkylaminomethyl)benzo[*h*]-1,6-naphthyridines. The nucleus and general substitution pattern of this molecule are derived by the "paper cyclization" of the chloroquine (I) side chain (as indicated by the arrow), along with aromatization of the formed third ring.



I



II

Consideration of the antimalarial activity found in the 4-quinolinemethanol series as well as in that of the phenanthrenemethanols[2,3] led to the refinement of replacing the formed dialkylaminomethyl group by a 2′-dialkylamino-1′-hydroxyethyl species at the 4 position of the benzo[*h*]-1,6-naphthyridine (II).

Synthesis of the candidate antimalarial required the presence of a usable functional group at the 4-position of the tricyclic heteroaromatic nucleus. Since a benzo[*h*]-1,6-naphthyridin-4-ol (**9**) possessed a requisite functionality, we sought a reaction which would provide this type intermediate in high yields. The acid-catalyzed Conrad–Limpach reaction between 4-aminoquinoline and acetoacetic ester has been reported to give high yields of 2-methylbenzo[*h*]-1,6-naphthyridin-4-ol.[4] Applying this procedure,[4] we were unable to isolate the desired benzo[*h*]-1,6-naphthyridin-4-ols or their possible quinolylcrotonate intermediates from the reactions of 4-aminoquinoline or 4-amino-7-chloroquinoline with acetoacetic or benzoylacetic esters. Similarly, these reactants did not provide the quinolylaminocrotonates under conditions which normally yield anilinocrotonates from anilines and acetoacetic ester.[5] The Doebner[6] and Niementowsky[7] reactions were equally ineffective in providing desired benzo[*h*]-1,6-naphthyridines from 4-aminoquinolines and ethyl 4-amino-7-chloroquinoline-3-carboxylate, respectively.

Although the quinolylaminocrotonate (**3**) was not obtained upon treatment of 4-amino-7-chloroquinoline (**5**) with ethyl β-ethoxy-*cis*-crotonate, somewhat encouraging results were obtained when 4,7-dichloroquinoline (**1**) was treated with ethyl 3-aminocrotonate (**2**) to give the crotonate in 20–30% yields. All attempts to obtain the benzo[*h*]-1,6-naphthyridin-4-ol by cyclization of **3** were unsuccessful.

In a manner similar to the EMME synthesis[8] of quinolines and benzo[*h*]-1,6-naphthyridines,[4,9] we prepared 8-chlorobenzo[*h*]-1,6-naphthyridin-4-ol (**9**) from diethyl ethoxymethylenemalonate (EMME) and 4-amino-7-chloroquinoline (**5**) according to Scheme I. This intermediate did not possess the desired ring substitution at the 2-position. Bromination of 8-chlorobenzo[*h*]-1,6-naphthyridin-4-ol (**9**) yielded the bromo derivative (**10**) which was treated with CuCN to give the 4-cyano compound **11** (Scheme II). A small amount of DMSO was required to effect the aqueous alkaline hydrolysis of this nitrile to the acid **12**. The overall yield of the recrystallized acid (Table I) was 26%. The final product (**17**) was prepared from this acid using the general synthetic pathway of Lutz, *et al.*,[10] where similar quinolinemethanols were prepared.

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