Table IV—Cumulative Elimination of Radioactivity Due to Carbon-14 in Urine and Feces following Oral Administration of ^{14}C-Norgestimate in Combination with ^{3}H-Ethinyl Estradiol [a]

Cumulative Percent Dose Eliminated	Subject KB	LS	KK	Mean ± *SE*
During first 3 days	40.4	50.6	50.4	47.1 ± 3.4
During first 7 days	82.8	78.7	79.3	80.3 ± 1.3
During first 14 days	85.3	81.8	89.4	85.6 ± 2.1
In urine	46.4	35.0	49.4	43.6 ± 4.4
In feces	38.9	46.8	40.0	41.9 ± 2.5

[a] ^{14}C-Norgestimate (73.5 μCi, 0.49 mg) in combination with ^{3}H-ethinyl estradiol (103 μCi, 0.14 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsule.

Cumulative elimination of radioactivity in the seven subjects indicates that approximately half of the dose was excreted in the urine during the 14-day collection period, suggesting that a substantial fraction of the administrered radioactivity was absorbed. It cannot be assumed that the radioactivity recovered in the feces necessarily represents unabsorbed drug since studies with intravenously administered ^{14}C-norgestimate in dogs and rats demonstrated substantial biliary secretion of norgestimate and/or its metabolites (6). Estimates of the apparent half-life of radioactivity due to carbon-14 in the present study were consistent with those reported previously for dogs, rats, and monkeys (6).

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ACKNOWLEDGMENTS

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Orlando meeting, November 1976.

The authors thank Mr. Gerald F. Eisen and Mr. Timothy J. Kane for assistance.

Molecular Connectivity Study of Muscarinic Receptor Affinity of Acetylcholine Antagonists

LEMONT B. KIER *x and LOWELL H. HALL ‡

Received December 5, 1977, from the **Department of Pharmaceutical Chemistry, Medical College of Virginia, Richmond, VA 23298*, and the ‡*Department of Chemistry, Eastern Nazarene College, Quincy, MA 02170.* Accepted for publication February 3, 1978.

Abstract □ A correlation between three molecular connectivity indexes and the muscarinic receptor affinity of 104 acetylcholine antagonists was found. Analysis of structure from these indexes reveals not only the importance of the onium and the bulky portions of the molecule but also their virtual independence of each other on the affinity. Analysis of the onium group portion of the molecules indicates that its contribution to the experimental affinity is virtually constant through a variety of structural variations. The influence of the bulky side chains, in contrast, is quite structure dependent. The equation relating connectivity indexes to muscarinic affinity of antagonists is capable of predicting the affinity of other antagonists as well as a number of agonist molecules.

Keyphrases □ Molecular connectivity indexes—various acetylcholine antagonists, correlated to muscarinic receptor affinity □ Muscarinic receptor affinity—various acetylcholine antagonists, correlated to molecular connectivity indexes □ Acetylcholine antagonists, various—molecular connectivity indexes correlated to muscarinic receptor affinity □ Topological indexes—molecular connectivity correlated to muscarinic receptor affinity of various acetylcholine antagonists □ Structure–activity relationships—molecular connectivity indexes of various acetylcholine antagonists correlated to muscarinic receptor affinity

Since the early observation that atropine is a competitive antagonist of acetylcholine muscarinic action, many molecules have been synthesized in efforts to develop clinically useful drugs. The most potent agents possess an onium group (usually quaternary) and a relatively bulky moiety bridged to the nitrogen by a three- to five-carbon chain. This bulky moiety is presumed to increase the affinity of the molecule by interacting through van der Waals forces beyond the region of the muscarinic receptor (1). Some similarities between antagonists and acetylcholine structure were summarized (2).

BACKGROUND

Several studies were directed toward a definition of the structural similarities and differences between muscarinic agonists and antagonists. Burgen (3) suggested that the onium group plays a major role in drug–receptor interaction among agonists while the bulky side-chain moiety is the salient feature for activity among antagonists. Increased drug–receptor interaction at the bulky side chain presumably weakens the interaction at the onium group, giving rise to a diminished agonist but predominant antagonist effect.

Barlow *et al.* (4) tested agonist and antagonist molecules and concluded that changes in the structure of the onium group produce similar effects on affinity for both series. In contrast to Burgen's (3) proposal, this view advocates a comparable role for the onium group among agonists and antagonists.

A subsequent study (5) on more than 100 acetylcholine analogs revealed that changes in the molecule affinity as the onium group was modified were not entirely independent of the bulky side chain. However, the interdependence of onium and the bulky side chain was not marked. Other factors could be operating besides binding influences, including realignment at the receptor due to onium group changes, leading to altered affinity.

Goldstein *et al.* (6) stated that competitive antagonism could result from an interaction at a different receptor near the acetylcholine receptor (7). The antagonist–receptor interaction would modify the agonist re-

0022-3549/78/1000-1408$01.00/0

Table I—Calculated Antagonist Affinities and χ Variables for $R_1CH_2CH_2R_2$

Compound	R_1[a]	R_2[b]	$^4\chi_{PC}$	$^1\chi^v$	$^3\chi_C{}^v$	Log K Obs.	Log K Calc.	Log K Result
1	CH_3CH_2O	D	1.966	5.060	0.577	3.974	4.378	−0.404
2		G	1.436	5.059	0.757	4.72	4.221	0.351
3		H	1.655	5.646	0.577	4.007	4.297	−0.290
4	$CH_3CH_2CH_2$	C	1.832	4.896	0.757	4.399	4.476	−0.077
5		D	1.966	5.483	0.577	4.588	4.487	0.101
6		F	1.655	5.569	0.577	4.370	4.277	0.093
7		G	1.436	5.481	0.757	4.815	4.330	0.485
8		H	1.655	6.069	0.577	4.546	4.406	0.140
9	$C_6H_5CH_2COO$	A	1.527	5.328	1.451	4.533	5.290	−0.757
10		B	2.234	5.916	1.162	5.093	5.584	−0.491
11		C	2.609	6.503	0.933	5.379	5.710	−0.331
12		D	2.743	7.091	0.754	5.785	5.721	0.064
13		E	2.213	6.589	0.933	5.084	5.435	−0.351
14		F	2.433	7.176	0.754	5.568	5.511	0.057
15		G	2.213	7.089	0.933	5.194	5.564	−0.370
16		H	2.433	7.676	0.754	5.525	5.639	−0.114
17	$C_6H_5CH_2CH_2O$	A	1.183	5.355	1.392	4.702	4.960	−0.258
18		B	1.890	5.942	1.103	5.167	5.254	−0.087
19		C	2.265	6.530	0.875	5.415	5.380	0.035
20		D	2.399	7.118	0.695	5.758	5.391	0.367
21		E	1.869	6.616	0.875	5.224	5.105	0.119
22		F	2.088	7.203	0.695	5.431	5.181	0.250
23		G	1.869	7.116	0.875	5.369	5.234	0.135
24		H	2.088	7.703	0.695	5.507	5.310	0.197
25	$C_6H_5CH_2CH_2CH_2$	A	1.183	5.778	1.392	5.180	5.069	0.111
26		B	1.890	6.365	1.103	5.549	5.363	0.186
27		C	2.265	6.953	0.875	5.735	5.489	0.246
28		D	2.399	7.540	0.695	5.849	5.500	0.349
29		E	1.869	7.038	0.875	5.602	5.214	0.388
30		F	2.088	7.626	0.695	5.651	5.290	0.361
31		G	1.869	7.538	0.875	5.686	5.343	0.343
32		H	2.088	8.126	0.695	5.467	5.419	0.048
33	$C_6H_{11}CH_2COO$	A	1.527	6.289	1.537	5.067	5.653	−0.586
34		B	2.234	6.876	1.249	5.517	5.947	−0.430
35		C	2.609	7.464	1.020	5.569	6.073	−0.504
36		D	2.743	8.051	0.840	5.630	6.084	−0.454
37		E	2.213	7.549	1.020	5.433	5.798	−0.365
38		F	2.433	8.137	0.840	5.635	5.874	−0.239
39		G	2.213	8.049	1.020	5.429	5.927	−0.498
40		H	2.433	8.637	0.840	5.432	6.003	−0.571
41	$C_6H_{11}CH_2CH_2O$	A	1.183	6.315	1.478	5.282	5.323	−0.041
42		B	1.890	6.903	1.190	5.657	5.617	0.040
43		C	2.265	7.491	0.961	5.783	5.743	0.040
44		D	2.399	8.078	0.781	5.912	5.755	0.157
45		E	1.869	7.576	0.961	5.616	5.468	0.148
46		F	2.088	8.164	0.781	5.729	5.544	0.185
47		G	1.869	8.076	0.961	5.731	5.597	0.134
48		H	2.088	8.664	0.781	5.725	5.673	0.052
49	$C_6H_{11}CH_2CH_2CH_2$	A	1.183	6.738	1.478	5.387	5.432	−0.045
50		B	1.890	7.326	1.190	5.841	5.726	0.115
51		C	2.265	7.913	0.961	5.878	5.852	0.026
52		D	2.399	8.501	0.781	5.921	5.864	0.057
53		E	1.869	7.999	0.961	5.728	5.577	0.151
54		F	2.088	8.586	0.781	5.814	5.653	0.161
55		G	1.869	8.499	0.961	5.933	5.706	0.227
56		H	2.088	9.086	0.781	6.025	5.782	0.243
57	$(C_6H_5)_2CHCOO$	A	2.549	7.398	1.587	7.159	6.771	0.388
58		B	3.256	7.985	1.298	7.578	7.065	0.513
59		C	3.631	8.573	1.069	7.584	7.191	0.393
60		D	3.765	9.160	0.890	7.367	7.202	0.165
61		E	3.235	8.659	1.069	7.440	6.916	0.524
62		F	3.454	9.246	0.890	7.558	6.992	0.566
63		G	3.235	9.159	1.069	7.260	7.045	0.215
64		H	3.454	9.746	0.890	7.015	7.121	−0.106
65	$(C_6H_5)_2CHCH_2O$	A	2.131	7.398	1.569	6.413	6.433	−0.020
66		B	2.838	7.985	1.280	6.693	6.727	−0.034
67		C	3.213	8.573	1.051	6.543	6.853	−0.310
68		D	3.347	9.160	0.872	6.374	6.864	−0.490
69		E	2.816	8.659	1.051	6.507	6.578	−0.071
70		F	3.165	9.236	1.244	6.589	7.247	−0.658
71		G	2.816	9.159	1.051	6.182	6.707	−0.525
72		H	3.036	9.746	0.872	6.131	6.783	−0.652

(continued)

Table I—*Continued*

Compound	R_1[a]	R_2[b]	$^4\chi_{PC}$	$^1\chi^v$	$^3\chi_C^v$	Log K Obs.	Log K Calc.	Log K Result
73	$(C_6H_5)_2CHCH_2CH_2$	A	2.131	7.820	1.569	7.015	6.542	0.473
74		B	2.838	8.408	1.280	7.270	6.836	0.434
75		C	3.213	8.995	1.051	7.091	6.962	0.129
76		D	3.347	9.583	0.872	6.712	6.973	−0.261
77		E	2.816	9.081	1.051	6.788	6.687	0.101
78		F	3.036	9.669	0.872	6.858	6.763	0.095
79		G	2.816	9.581	1.051	6.664	6.816	−0.152
80		H	3.036	10.169	0.872	6.579	6.892	−0.313
81	$(C_6H_5)_2C(OH)COO$	A	4.313	7.505	1.713	8.511	8.288	0.223
82		B	5.020	8.093	1.424	8.934	8.582	0.352
83		C	5.395	8.680	1.195	8.957	8.708	0.249
84		D	5.529	9.268	1.016	8.682	8.719	−0.037
85		E	4.998	8.766	1.195	8.585	8.433	0.152
86		F	5.218	9.354	1.016	8.652	8.509	0.143
87		G	4.998	9.266	1.195	8.034	8.562	−0.528
88		H	5.218	9.854	1.016	8.012	8.638	−0.626
89	$(C_6H_{11})_2CHCOO$	A	2.549	9.299	1.752	7.686	7.482	0.204
90		B	3.256	9.886	1.463	7.723	7.776	−0.053
91		C	3.631	10.474	1.234	8.083	7.902	0.181
92		D	3.765	11.061	1.055	8.068	7.913	0.155
93		E	3.235	10.560	1.234	8.093	7.627	0.466
94		F	3.454	11.147	1.055	8.260	7.703	0.557
95		G	3.235	11.060	1.234	8.117	7.756	0.361
96		H	3.454	11.647	1.055	7.692	7.832	−0.140
97	$(C_6H_{11})CHCH_2O$	A	2.131	9.299	1.744	7.254	7.158	0.096
98		B	2.838	9.886	1.455	7.615	7.452	0.163
99		C	3.213	10.474	1.226	7.574	7.578	−0.004
100		D	3.347	11.061	1.047	7.354	7.589	−0.235
101		E	2.816	10.560	1.226	7.541	7.303	0.238
102		F	3.036	11.147	1.047	7.206	7.379	−0.173
103		G	2.816	11.060	1.226	7.296	7.432	−0.136
104		H	3.036	11.647	1.047	6.600	7.508	−0.908

[a] The molecular formula for each R_1 group is given only for the first compound of each set. It is understood that the subsequent compounds have the same R_1, until a new formula is given. [b] Symbols for the onium groups are as follows: A, $N(CH_3)_3$; B, $N(CH_3)_2C_2H_5$; C, $N(C_2H_5)_2CH_3$; D, $N(C_2H_5)_3$; E, $N(CH_2)_4CH_3$; F, $N(CH_2)_4C_2H_5$; G, $N(CH_2)_5CH_3$; and H, $N(CH_2)_5C_2H_5$.

ceptor, thereby reducing the affinity of the agonist–receptor interaction.

Aside from the question of the interrelation of onium and the side chain in determining affinity, the question of the overall structure–activity effects on affinity has not been evaluated quantitatively. Relationships between affinity and molar volume, osmotic coefficients, and ion-pair formation energies were studied (7–9). Parallel physical properties were considered, leading to the general conclusion that size influences hydrophobic bonding, resulting in increased measured affinity.

EXPERIMENTAL

The work of Abramson *et al.* (5) on a large series of antagonists and agonists provides the best data for a quantitative structure–activity study and a consideration of some conflicting views. Qualitative observations on the structure–activity relationships of more than 100 compounds, mostly antagonists, were made relative to the affinities at the guinea pig ileum.

In the present study, 104 optically inactive antagonists reported by Abramson *et al.* (5) were considered. The structural analysis was made using the molecular connectivity method (10); this method was employed previously (11–15) to study the structure–activity relationships of various biologically active molecules.

RESULTS

The connectivity indexes for the 104 antagonist molecules were searched, using regression analysis, for the best three indexes correlating with affinity (expressed as log K). The best equation was:

$$\log K = 0.749\ (\pm 0.002)\,{}^4\chi_{PC} + 0.258\ (\pm 0.001)\,{}^1\chi^v + 1.340\ (\pm 0.015)\,{}^3\chi_C^v + 0.827\ (\pm 0.33) \quad \text{(Eq. 1)}$$

$$r = 0.962 \quad s = 0.331 \quad n = 104 \quad F = 417 \quad p < 0.001$$

The equation accounts for 93% of the variation in log K, and the standard deviation is less than 7% of the range of log K values in the study (Table I). Only two molecules, Compounds 9 and 104, are predicted by the equation to have a residual greater than 2 *SD*. The correlation matrix is shown in Table II. The indexes used in the regression search include $^0\chi$, the path terms of orders 1 through 4, $^3\chi_C$, $^4\chi_{PC}$, and the corresponding valence terms.

The three indexes in the equation are weighted counts of subgraphs of a specific type. The $^1\chi^v$ index refers to subgraphs of one bond in which the bridged atoms are described by their valence, δ^v, according to the prescription $\delta^v = Z^v - h$, where Z^v is the number of valence electrons in the atom and h is the number of hydrogens bonded to the atom but suppressed in forming the graph. The $^3\chi_C^v$ index describes subgraph fragments in which three bonds converge at one atom. The atoms are described by their δ^v in the computation. The $^4\chi_{PC}$ index quantitates the subgraph fragments that would be identical to the isopentane molecular skeleton. The atom δ values are based on adjacency.

The first two indexes in the equation cross correlate to an extent of 0.65. This low order is not negligible but arises as a consequence of the nature of the molecules under study. Increases in $^1\chi^v$ generally arise from an increase in the number of atoms in the molecule. Increases in the $^4\chi_{PC}$ index reflect an increase in branching in the molecule. In the set of molecules chosen, these structural changes parallel each other to the extent reflected by the cross-correlation value of 0.65. The change in one index accounts for 42% of the variation in the other index.

The indexes in the equation describe specific subgraphs irrespective of their degree of statistical cross correlations. In a multiple index relationship for a structurally similar set of molecules, this result is not unexpected. Nevertheless, the relative amount of information conveyed

Table II—Correlation Matrix for Equation Variables

	$^1\chi^v$	$^4\chi_{PC}$
$^1\chi^v$		
$^4\chi_{PC}$	0.65	
$^3\chi_C^v$	0.25	0.20

Table III—Connectivity Indexes and Log *K* Contributions of Onium Group Models

Group Model	$^4\chi_{PC}$ Analysis n^a	$^4\chi_{PC}$	Average	$^1\chi^v$	$^3\chi_C{}^v$ Analysis n	$^3\chi_C{}^v$	Average	Calculated Log *K* Contribution
$C_2H_5N(CH_3)_3$	3	1.061	0.354	2.221	4	1.274	0.319	3.902
$C_2H_N(CH_3)_2C_2H_5$	6	1.707	0.285	2.808	4	0.986	0.247	4.151
$C_2H_5N(CH_3)(C_2H_5)_2$	9	2.030	0.226	3.396	4	0.757	0.189	4.238
$C_2H_5N(C_2H_5)_3$	12	2.121	0.177	3.983	4	0.577	0.144	4.216
$C_2H_5N(CH_2)_4CH_3$	9	1.634	0.182	3.481	4	0.757	0.189	3.963
$C_2H_5N(CH_2)_4C_2H_5$	12	1.811	0.151	4.069	4	0.577	0.144	4.006
$C_2H_5N(CH_2)_5CH_3$	9	1.634	0.182	3.981	4	0.757	0.189	4.092
$C_2H_5N(CH_2)_5C_2H_5$	12	1.811	0.151	4.569	4	0.577	0.144	4.135

[a] n = number of terms.

by each index in a successful correlation with a property affords an opportunity for meaningful structural analysis and possible prediction.

The equation is statistically significant above the 99.9% level; $F = 416.5 > 6.04 = F(100,3,0.001)$. The addition of the second variable, $^4\chi_{PC}$, to the one-variable equation based on $^1\chi^v$ is statistically significant above the 99.9% level; $F = 145.2 > 11.9 = F(101,1,0.001)$. So also is the addition of the third variable, $^3\chi_C{}^v$; $F = 121.1 > 11.8 = F(100,1,0.001)$.

Random Number Analysis—When a regression equation is obtained from a search of several variables, a question can be raised about possible random correlations (16). In this study, this question was approached with a direct investigation of correlations based on replacement of the 22 χ terms with random numbers. The regression search program was used to find the best set of three variables against the affinity data in the same manner as was done using χ indexes. For each set of random numbers, 1540 triplets of variables were considered. The highest correlation coefficient, r_{max}, was recorded. The procedure was repeated 100 times with different sets of random numbers for all 22 variables. The highest r_{max} value found was 0.45, and the average was 0.25. Four values of r_{max} were in the range 0.35–0.45, 35 were between 0.25 and 0.35, 60 were between 0.15 and 0.25, and one was below 0.15.

In a further study, the three χ terms found to correlate with log *K*, $^4\chi_{PC}$, $^1\chi^v$, and $^3\chi_C{}^v$, were regressed against 100 sets of random numbers in place of log *K*. The highest correlation coefficient found was 0.35, and the average value was 0.16. Ten values lay in the range 0.25–0.35, 43 lay between 0.15 and 0.25, and 47 were between 0.05 and 0.15.

These studies clearly indicate that the set of three χ terms reported here do not correlate with sets of random numbers. Rather, the three terms in concert reflect the systematic variation of log *K* with the molecular structure. In addition, the χ terms yield a regression vastly superior to sets of random numbers. These results further support the conclusion that the correlation reported here is a systematic relation.

Analysis—The best variables as shown in the equation of correlation are no longer than three path lengths (three contiguous bonds as found in the $^4\chi_{PC}$ subgraphs). Indexes reflecting subgraphs through four path lengths were searched in the regression analysis. Additional regression analyses revealed no significant role for indexes based on paths of length five and six.

Among these structures, this finding leads to the conclusion that important structural features may be found at either end of the molecule and are not connected through the length of the molecule. Molecular connectivity provides a partial statement of the real structure of these molecules since the conformation is not dealt with by this method. Thus, the separate ends of the molecule, revealed by molecular connectivity to be important, may in fact be physically quite close because of forces influencing the conformational preference of the molecule. Molecular connectivity speaks to sequential attachment of atoms through formal bonds, not transspatial nonbonded forces and interactions.

The finding of a separate role for the two ends of the molecule, in the sense just described, prompted an additional study to analyze separately the onium group and the side chain for further structural information.

Onium Group—For convenience in analyzing the eight onium group variants, a truncated model of the onium groups in which the bulky side chain was replaced in each case by an ethyl group was used. Table III lists the values of the three connectivity indexes, the number of subgraph terms contributing to each index, and the average value of the subgraphs. The data illustrate a number of structure–activity relationship conclusions about the onium groups and affinity.

The first four onium groups in Table III are a homologous series formed by successively replacing an *N*-methyl group with an ethyl group. The number of subgraphs composing the $^4\chi_{PC}$ index increased by three through this series. However, the numerical values of the subgraph contributions, as illustrated by the average value, decreased in this progression. The net effect was a modest increase in the value of $^4\chi_{PC}$ after the second compound.

The $^3\chi_C{}^v$ terms reveal a different trend in Table III for the first four cases. The number of subgraph terms composing this index in each case was constantly four, while the average value declined numerically. Thus, the $^3\chi_C{}^v$ indexes decreased with successive ethyl group replacement.

The $^1\chi^v$ indexes increased through this series, reflecting the addition of one carbon atom in each case. Thus, a summation of these three connectivity indexes, weighted by the coefficients in the regression equation, reveals a contribution to the log *K* values from the onium group. As can be seen in the last column of Table III, this contribution was quite similar across the series. The numerical range for the first four cases (and indeed all eight cases) was within the standard deviation of the equation.

An analysis of all eight onium group models in Table III gives the same conclusion. The contributions to log *K* from increasing the number of atoms was offset by a decline in the $^3\chi_C{}^v$ terms and a leveling out of the values of the $^4\chi_{PC}$ terms. The net result was a relatively constant contribution to log *K* by the onium group, irrespective of the increase in size or complexity.

Side Chain—The bulky side chain was treated similarly by analyzing the individual connectivity indexes for truncated models of the side chains found in this study. The truncation model utilized an ethyl group

Table IV—Connectivity Indexes and Log *K* Contributions of Side-Chain Models

Group Model	$^4\chi_{PC}$ Analysis n^a	$^4\chi_{PC}$	Average	$^1\chi^v$	$^3\chi_C{}^v$ Analysis n	$^3\chi_C{}^v$	Average	Calculated Log *K* Contribution
$CH_3CH_2OC_2H_5$	0	0	0	1.992	0	0	0	1.341
$CH_3CH_2CH_2C_2H_5$	0	0	0	2.414	0	0	0	1.450
$C_6H_5CH_2COOC_2H_5$	5	0.777	0.155	4.022	2	0.177	0.089	2.684
$C_6H_5CH_2CH_2OC_2H_5$	3	0.433	0.144	4.049	1	0.118	0.118	2.354
$C_6H_5CH_2CH_2CH_2C_2H_5$	3	0.433	0.144	4.471	1	0.118	0.118	2.463
$C_6H_{11}CH_2COOC_2H_5$	5	0.777	0.155	4.982	2	0.263	0.132	3.047
$C_6H_{11}CH_2CH_2OC_2H_5$	3	0.433	0.144	5.009	1	0.204	0.204	2.717
$C_6H_{11}CH_2CH_2CH_2C_2H_5$	3	0.433	0.144	5.432	1	0.204	0.204	2.826
$(C_6H_5)_2CHCOOC_2H_5$	17	1.799	0.106	6.091	4	0.313	0.078	4.165
$(C_6H_5)_2CHCH_2OC_2H_5$	13	1.381	0.106	6.091	3	0.295	0.098	3.828
$(C_6H_5)_2CHCH_2CH_2C_2H_5$	13	1.381	0.106	6.514	3	0.295	0.098	3.937
$(C_6H_5)_2C(OH)COOC_2H_5$	32	3.563	0.111	6.199	7	0.439	0.063	5.683
$(C_6H_{11})_2CHCOOC_2H_5$	17	1.799	0.106	7.992	4	0.478	0.120	4.877
$(C_6H_{11})_2CHCH_2OC_2H_5$	13	1.381	0.106	7.992	3	0.469	0.156	4.552

[a] n = number of terms.

Table V—Prediction of Antagonist Affinities

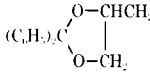
Molecule	$^4\chi_{PC}$	$^1\chi^v$	$^3\chi_C{}^v$	Experimental Log K	Calculated Log K
$C_6H_5(CH_2)_4N(CH_3)_3$	1.183	5.278	1.392	4.771	4.949
$C_6H_5(CH_2)_6N(CH_3)_3$	1.183	6.278	1.392	5.393	5.198
$C_6H_5(CH_2)_4N(C_2H_5)_3$	2.399	7.040	0.695	5.480	5.371
$C_6H_5(CH_2)_6N(C_2H_5)_3$	2.399	8.040	0.695	5.970	5.629
	3.541	7.584	1.744	8.020	7.773

Table VI—Prediction of Agonist Affinities

Molecule	$^4\chi_{PC}$	$^1\chi^v$	$^3\chi_C{}^v$	Experimental Log K	Calculated Log K
$C_2H_5OCH_2CH_2N(CH_3)_3$	0.750	3.298	1.274	4.074	3.947
$C_2H_5OCH_2CH_2N(CH_3)_2C_2H_5$	1.457	3.885	0.986	3.887	4.242
$C_2H_5OCH_2CH_2CH_2N(C_2H_5)_2CH_3$	1.832	4.473	0.757	3.735	4.368
$C_2H_5OCH_2CH_2N(CH_2)_4CH_3$	1.436	4.559	0.757	4.026	4.093
$C_2H_5OCH_2CH_2N(CH_2)_4C_2H_5$	1.665	5.146	0.577	3.883	4.167
$C_2H_5CH_2CH_2CH_2N(CH_3)_3$	0.750	3.721	1.274	3.733	4.056
$C_2H_5CH_2CH_2CH_2N(CH_3)_2C_2H_5$	1.457	4.308	0.986	3.970	4.351
$C_2H_5CH_2CH_2CH_2N(CH_2)_4CH_3$	1.436	4.981	0.757	4.165	4.202

terminating the molecule in place of the ethylonium group found in each actual compound. The data for analysis are summarized in Table IV.

The major variation of affinity with molecular structure was associated with side-chain variation (Table IV). This influence was paralleled by all three connectivity indexes in Table IV. The major fraction of the variation was described by the $^1\chi^v$ contribution. The range of $^1\chi^v$ contributions was about 2 log K units while the contributions from $^3\chi_C{}^v$ and $^4\chi_{PC}$ ranged over 0.5 and 0.7 log K unit, respectively.

A more specific analysis of the data can be made in terms of structural features contributing to affinity. The replacement of methylene by an ether oxygen decreased the value of $^1\chi^v$ by 0.422. This difference reflected the observations that the ether side chains had a slightly lower affinity than the alkyl side chains. Cyclohexyl substituents gave $^1\chi^v$ values 0.96 higher than phenyl substituents, mirroring the trend in affinities.

The replacement of a methyl hydrogen in the hydrocarbon or ether series by a phenyl or cyclohexyl increased the affinity significantly. This effect was described principally by the increase in $^1\chi^v$. However, when both groups appeared on the same carbon, additional $^4\chi_{PC}$ and $^3\chi_C{}^v$ subgraphs were generated, giving an increase in affinity greater than anticipated based on the individual contributions. This observation was properly accounted for by Eq. 1 and corresponded to a reported observation (5), but here it was in quantitative form.

Prediction of Antagonist Affinities—The value of the structural descriptions encoded in Eq. 1 lies in the ability to predict the affinities of antagonists not included in the original regression analysis. Accordingly, the χ indexes were calculated for five additional antagonists and the log K values were computed from Eq. 1 (Table V). The predicted values of the affinities were within the standard deviation of the equation.

Prediction of Agonist Affinities—As a further test of Eq. 1 and to determine whether comparable structural features govern the affinities of agonists, Eq. 1 was used to predict the affinities of five agonists and three partial agonists. The results (Table VI) reveal good predictions in almost every case. The standard deviation was within the standard deviation of the original equation.

DISCUSSION

Based on a quantitative structure–activity relationship accounting for 93% of the variation in the log K values for 104 molecules, certain general conclusions about structure and affinity can be made. The affinity of the antagonists in Table I depends on a specific combination of molecular size, branching, and heteroatom composition.

The onium group contribution to the affinity of the molecule is essentially constant, despite structural changes such as size and cyclization (Table III). The variation in the onium group contribution to log K ranges from 3.902 to 4.238 for the eight variations in this study. This range coincides with the standard deviation in the equation.

The same kind of analysis applied to the onium groups of the agonists in Table VI also reveals a fairly constant contribution to the log K values. Furthermore, these onium group contributions are the same as those found among the antagonists. This finding is quite compatible with the views of Barlow *et al.* (4).

The principal influence on the affinity of antagonists arises from structural variation in the side chain. This finding is evident from Table V, which depicts the contributions from 14 different side chains in the study.

The close predictions for antagonists and agonists in Tables V and VI indicate that the molecular connectivity indexes in the equation correctly describe the structural influences governing the affinity.

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