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AN ANALYSIS OF THE STRUCTURE–ACTIVITY RELATIONSHIP IN THE ADRENERGIC BLOCKING ACTIVITY OF THE β-HALOALKYLAMINES*

CORWIN HANSCH† and ERIC J. LIEN

Department of Chemistry, Pomona College, Claremont, Calif., U.S.A.

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Abstract—The work of Hall on the correlation of base strengths of aliphatic amines using Taft's polar constant, σ^*, has been extended. It has been shown that the p*Ka* values for 92 amines can be correlated in a single two-parameter equation using σ^* and n_H, where n_H indicates the number of hydrogen atoms on the protonated amino nitrogen. This relation can then form the basis for correlating chemical structure and biological activity of amines. In this paper the antagonism of adrenaline, noradrenaline and 5-hydroxytryptamine (5-HT) by a variety of 2-bromo-2-phenylalkylamines is considered. It is shown that substitutents on the aromatic ring are involved in hydrophobic bonding, while those on nitrogen do not seem to influence the biological activity in this fashion. The function of the nitrogen substituents appears to be limited to that of regulating the availability of the lone-pair electrons for immonium ion formation and for its reactivity. The compactness of the nitrogen substituents is shown to be necessary for high adrenergic blocking activity. Possible ways of molecular modification for more active compounds are suggested.

SINCE the report of Nickerson and Goodman[1] in 1947 on the adrenergic blocking properties of Dibenamine, hundreds of structurally related compounds have been synthesized and tested.[2–8] The accumulated data have led to the establishment of certain structural requirements that are considered to be necessary for activity.[2, 9–11] Much of the discussion has been focusing on the mechanism of action via the ethylene immonium ion.[4, 10–12] The purpose of this report is to analyze, in quantitative terms, the effect of substituents on the biological activity according to our previously reported[13–15] extra-thermodynamic[16] method. Biological activity is discussed in terms of our hydrophobic bonding constant,[17–19] π, the Hammett[20] constant, σ, the Taft polar constant,[17–19] σ^*, and Hancock's[21] steric parameter, E_s^c.

METHOD

The biological data[7, 22] are summarized in Tables 1–4. The substituent constants[16–19, 21] are given in Tables 1 and 3.

Relatively little quantitative work has been published about the use of the parameters σ^* and E_s^c with groups on nitrogen. The work of Hall[23] does establish the

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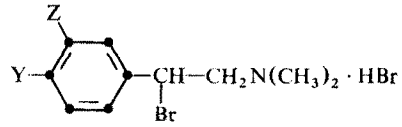
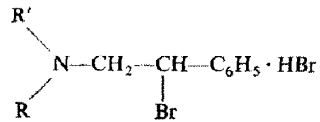
TABLE 1. ANTAGONISM OF *N*,N-DIMETHYL-2-BROMOPHENETHYLAMINES TO ADRENALINE AND NORADRENALINE



			Log 1/C in the rat							
			vs. Adrenaline			vs. Noradrenaline				
	Y	Z	Obsd*	Calcd†	Δ	Obsd*	Calcd‡	Δ	Σπ§	Σσ‖
1.	H	H	7·46	7·89	−0·43	7·25	7·85	−0·60	0·00	0·00
2.	F	H	8·16	7·98	0·18	8·13	7·94	0·19	0·15	0·06
3.	Cl	H	8·68	8·38	0·30	8·68	8·36	0·32	0·70	0·23
4.	Br	H	8·89	8·77	0·12	8·89	8·76	0·13	1·02	0·23
5.	I	H	9·25	8·98	0·27	9·29	8·98	0·31	1·26	0·28
6.	Me	H	9·30	8·79	0·51	9·28	8·78	0·50	0·52	−0·17
7.	H	F	7·52	7·51	0·01	7·50	7·46	0·04	0·13	0·34
8.	H	Cl	8·16	8·23	−0·07	8·15	8·21	−0·06	0·76	0·37
9.	H	Br	8·30	8·42	−0·12	8·30	8·40	−0·10	0·94	0·39
10.	H	I	8·40	8·74	−0·34	8·40	8·73	−0·33	1·15	0·35
11.	H	Me	8·46	8·62	−0·16	8·52	8·60	−0·08	0·51	−0·07
12.	F	Cl	8·19	8·32	−0·13	8·19	8·30	−0·11	0·91	0·43
13.	F	Br	8·57	8·51	0·06	8·57	8·49	0·08	1·09	0·45
14.	F	Me	8·82	8·71	0·11	8·82	8·69	0·13	0·66	−0·01
15.	Cl	Cl	8·89	8·72	0·17	8·89	8·72	0·17	1·46	0·60
16.	Cl	Br	8·92	8·91	0·01	8·92	8·91	0·01	1·64	0·62
17.	Cl	Me	8·96	9·11	−0·15	8·96	9·11	−0·15	1·21	0·16
18.	Br	Cl	9·00	9·11	−0·11	9·00	9·12	−0·12	1·78	0·60
19.	Br	Br	9·35	9·30	0·05	9·30	9·32	−0·02	1·96	0·62
20.	Br	Me	9·22	9·49	−0·27	9·22	9·50	−0·28	1·53	0·17
21.	Me	Me	9·30	9·53	−0·23	9·30	9·53	−0·23	1·03	−0·24
22.	Me	Br	9·52	9·32	0·20	9·52	9·33	0·19	1·46	0·22
23.	Ph	H	7·52	10·18¶	−2·66	7·52	10·21¶	−2·70	1·89	0·01

* From ref. 22. C is the ED_{50} in mole/kg.
† Calculated from Eq. 7.
‡ Calculated from Eq. 11.
§ π values are taken from the phenoxyacetic acid system, ref. 18.
‖ From ref. 20.
¶ These points were not used in the regression analysis.

TABLE 2. ADRENERGIC BLOCKING ACTIVITY OF N-SUBSTITUTED 2-BROMOALKYLAMINES



			Log 1/C in the cat						Log 1/C in mice		
			vs. Adrenaline			vs. Noradrenaline			vs. Adrenaline		
	R	R'	Obsd*	Calcd†	Δ	Obsd*	Calcd‡	Δ	Obsd*	Calcd§	Δ
24.	CH_3	CH_3	7·52	7·48	0·04	7·40	7·37	0·03	5·85	5·92	−0·07
25.	C_2H_5	C_2H_5	5·57	5·92	−0·35	5·12	5·75	−0·63	4·82	5·17	−0·35
26.	*n*-C_3H_7	*n*-C_3H_7	5·37	5·19	0·18	5·26	4·99	0·27	5·20	4·92	0·28
27.	*i*-C_3H_7	*i*-C_3H_7	3·70	3·72	−0·02	3·62	3·49	0·13			
28.	*n*-C_4H_7	*n*-C_4H_9	5·12	4·99	0·13	5·00	4·80	0·20	4·96	4·82	0·14
29.	CH_3	H	5·26	5·15	0·11	5·62	5·13	0·49	4·89	4·30	0·59
30.	C_2H_5	H	4·59	4·37	0·22	4·70	4·32	0·38	3·59	3·92	−0·33
31.	*n*-C_3H_7	H	3·68	3·98	−0·30	3·55	3·92	−0·37	3·80	3·79	0·01
32.	*n*-C_4H_9	H	3·92	3·91	0·01	3·44	3·85	−0·41	3·59	3·75	−0·16
33.	$C_6H_5CH_2$	H	5·12	5·17	−0·05	5·11	5·20	−0·09	4·60	4·70	−0·10

* From ref. 7. C is the ED_{50} in mole/kg.
† Calculated from Eq. 15.
‡ Calculated from Eq. 18.
§ Calculated from Eq. 20.

TABLE 3. ANTIADRENALINE POTENCIES OF *N*-SUBSTITUTED 2-BROMOALKYLAMINES ON RAT BLOOD PRESSURE*

			Log 1/C									
			vs. Adrenaline			vs. Noradrenaline						
	R	R′	Obsd.†	Calcd‡	Δ	Obsd.†	Calcd‡	Δ	ΣE_s[c]	n_H	$\Sigma\pi$	$\Sigma\sigma$*
24.	CH_3	CH_3	7·46	6·78	0·68	7·25	6·69	0·56	0·00	1	1·00	0·00
25.	C_2H_5	C_2H_5	5·29	5·92	−0·63	5·75	5·93	−0·18	−0·76	1	2·00	−0·20
26.	*n*-C_3H_7	*n*-C_3H_7	6·00	5·37	0·63	5·89	5·44	0·45	−1·34	1	3·00	−0·23
27.	*i*-C_3H_7	*i*-C_3H_7	4·52	4·52	0·00	4·66	4·67	−0·01	−2·16	1	2·60	−0·38
28.	*n*-C_4H_9	*n*-C_4H_9	5·36	5·28	0·08	5·35	5·37	−0·02	−1·40	1	4·00	−0·26
29.	CH_3	H	6·22	4·39‖	1·83	6·30	4·36‖	1·94	0·32	2	0·50	0·49
30.	C_2H_5	H	4·00	3·97	0·03	4·10	3·98	0·12	−0·06	2	1·00	0·39
31.	*n*-C_3H_7	H	3·60	3·69	−0·09	3·60	3·73	−0·13	−0·35	2	1·50	0·37
32.	*n*-C_4H_9	H	3·70	3·65	0·05	3·70	3·70	0·00	−0·38	2	2·00	0·36
33.	$C_6H_5CH_2$	H	5·52	3·95‖	1·57	5·55	4·56‖	0·99	−0·37	2	2·63	0·71
34.	C_2H_5	CH_3	5·55	6·35	−0·80	5·52	6·31	−0·79	−0·38	1	1·50	−0·10
35.	CH_3	$C_6H_5CH_2$	6·66	6·34	0·32	6·41	6·29	0·12	−0·69	1	3·13	0·22
36.	$C_6H_5CH_2$	$C_6H_5CH_2$	5·62	5·89	−0·27	5·77	5·89	−0·11	−1·38	1	5·26	0·43

* For structural formulas, see Table 2.
† From ref. 7. C is the ED_{50} in mole/kg.
‡ Calculated from Eq. 21.
§ Calculated from Eq. 22.
‖ These points were not used in the regression analysis.

TABLE 4. ANTAGONISM TO 5-HT BY N-SUBSTITUTED 2-BROMOALKYLAMINES*

			Log 1/C					
			On isolated rat uterus			On guinea-pig ileum		
	R	R′	Obsd†	Calc.‡	Δ	Obsd.†	Calc.§	Δ
24.	CH_3	CH_3	3·46	3·54	−0·08			
25.	C_2H_5	C_2H_5	2·72	2·68	0·04	1·23	1·18	0·05
26.	n-C_3H_7	n-C_3H_7	2·72	2·54	0·18	1·00	0·77	0·23
28.	n-C_4H_9	n-C_4H_9	2·27	2·41	−0·14	0·30	0·59	−0·29
29.	CH_3	H	2·06	1·97	0·09	1·62	1·74	−0·12
30.	C_2H_5	H	1·59	1·54	0·05	1·13	1·05	0·08
31.	n-C_3H_7	H	1·51	1·44	0·07	1·08	0·82	0·26
32.	n-C_4H_9	H	1·19	1·40	−0·21	0·52	0·75	−0·23

* For structural formulas see Table 2.
† From ref. 7. C is the ED_{50} in mole/l.
‡ Calculated from Eq. 23.
§ Calculated from Eq. 24.

fact that good correlations of pK_a of aliphatic amines can be obtained by using σ^*. He has shown that good correlations such as those shown in Eqs 1, 2 and 3 can be obtained. We have re-derived Eq. 1, 2 and 3 by using Hall's collection of amines plus the following, with pK_a values in parentheses: n-octylamine[24] (10·65); $N{\equiv}CCH_2NH_2$[24] (5·34); isoamylamine[25] (10·70); diisoamylamine[25] (10·98); triisobutylamine[25] (10·42); neopentyl-amine[26] (10·38); n-amylamine[27] (10·74); ethyl isopropyl amine[27] (11·13); methyldi-n-propylamine[28] (10·40); methyldi-n-amylamine[28] (10·40); methyl-n-butyl-n-amylamine[28] (10·40); methyldi-n-butylamine[28] (10·50); methyl-n-propyl-n-amylamine[28] (10·40); methyldiisobutylamine[28] (10·10); methyldi-*sec*-butylamine[28] (11·10); methylisopropyl-*tert*-amylamine[28] (11·20); methyl-*tert*-butyl-*tert*-amylamine[28] (11·90).

		n	r	s	
Primary	$pK_a = -3{\cdot}201\sigma^* + 13{\cdot}214$	27	0·989	0·257	(1)
Secondary	$pK_a = -2{\cdot}931\sigma^* + 11{\cdot}631$	22	0·986	0·316	(2)
Tertiary	$pK_a = -3{\cdot}243\sigma^* + 9{\cdot}558$	43	0·995	0·165	(3)

In the above equations, n is the number of data points used in the regression, s is the standard deviation, and r is the correlation coefficient. As Hall pointed out, the slopes of eqs 1–3 are quite close so that the difference in the intercepts can be attributed to the number of hydrogen atoms on nitrogen. The constants in our Eqs 1–3 are slightly different from Hall's due to the inclusion of new data. Equations 1–3 can be integrated into Eq. 4.

	n	r	s	
$pK_a = -3{\cdot}140\sigma^* + 1{\cdot}816n_H + 7{\cdot}817$	92	0·985	0·299	(4)

In Eq. 4, n_H represents the number of hydrogens on the protonated amine, 3 for primary, 2 for secondary, etc. One can compare the goodness of fit of Eqs 1–4 by comparing values of s. It is seen that little is lost in precision of fit for primary and secondary amines. The error for tertiary is greater but not serious for the purpose of correlating biological activity. Actually, the rather poor fit of Eq. 2 may be due more to error in pK_a values than in σ^* constants.

We feel the fact that base strength of the three classes of amines can be correlated in one equation justifies the use of this approach in the analysis of biochemical problems. In such problems steric effects may often be greater than reaction with a small acid and it may be necessary to include E_s.

RESULTS

From the data in Table 1 for the effect of the β-haloamines vs. adrenaline in rats we have derived, via the method of least squares, Eqs 5–8. In the 22 derivatives considered, only substituents on the ring were varied; the side chain was held constant.

	n	r	s	
$\log\frac{1}{C} = 0{\cdot}770\pi + 7{\cdot}931$	22	0·724	0·402	(5)
$\log\frac{1}{C} = -0{\cdot}034\sigma + 8{\cdot}704$	22	0·022	0·583	(6)
$\log\frac{1}{C} = 1{\cdot}221\pi - 1{\cdot}587\sigma + 7{\cdot}888$	22	0·918	0·238	(7)
$\log\frac{1}{C} = -0{\cdot}282\pi^2 + 1{\cdot}537\sigma - 0{\cdot}316\sigma^2 - 2{\cdot}134\sigma + 0{\cdot}746\,(\pi\sigma) + 7{\cdot}862$	22	0·924	0·249	(8)

Comparing Eqs 5 and 6, it is seen that the more important character of the substituent in affecting the biological activity is its hydrophobic bonding ability. Equation 7 shows that electronic effects do play a significant role. The roles of π and σ appear to be linear and additive since Eq. 8 does not give a better correlation than Eq. 7 (compare values of s). The poor fit of compound 23 may be due to a steric effect of the large phenyl group in the 4-position preventing the drug from proper fit on the receptors.

Equations 9–11 result from the data of haloamines vs. noradrenaline.

	n	r	s	
$\log\frac{1}{C} = 0{\cdot}802\pi + 7{\cdot}888$	22	0·724	0·525	(9)
$\log\frac{1}{C} = -0{\cdot}014\sigma + 8{\cdot}689$	22	0·014	0·607	(10)
$\log\frac{1}{C} = 1{\cdot}263\pi - 1{\cdot}620\sigma + 7{\cdot}845$	22	0·911	0·257	(11)

Again, going to a higher order equation like Eq. 8 does not result in an improved correlation. The constants and goodness of fit shown by Eq. 7 and Eq. 11 are surprisingly close and testify to the high quality of the biological tests. The positive coefficient associated with π indicates that more lipophilic substituents would give more active derivatives. The fact that compound 23 is poorly predicted might mean that it is too lipophilic. This, however, is unlikely since compound 19 has a $\Sigma\pi$ of 1·96 and fits well. $\Sigma\pi$ for compound 23 is only 1·89. Hence, a steric factor appears to be the reason for the poor fit of 23. This might be circumvented by moving large

groups to the 3- or 5-position or to both. The fact that σ has a negative coefficient means that electron-releasing groups are more effective in promoting activity and thus in this sense halogens are not the best choice for activating substituents. It would seem to us that alkoxy grops in the 3- and 5-positions should lead to more active compounds. Since these are rather readily dealkylated in the body by what appears[29] to be an attack on the hydrogen on the alpha carbon atom, the tertiary butyl and tertiary amyl groups would be most interesting to explore. Dialkylamino groups could also be designed to resist dealkylation and have high π values. It must be borne in mind that since π is, to a certain extent, dependent on σ, some of the effect of σ in Eqs 5–11 may be associated with partitioning effects.[18]

From the data in Table 2 we have derived Eqs 12–17 for the antagonism of adrenaline in the cat.

	n	r	s	
$\log\frac{1}{C} = 0{\cdot}482E_s^c + 5{\cdot}299$	10	0·325	1·143	(12)
$\log\frac{1}{C} = -0{\cdot}356\pi - 0{\cdot}921\sigma^* + 5{\cdot}821$	10	0·319	1·224	(13)
$\log\frac{1}{C} = 1{\cdot}666E_s^c - 3{\cdot}032\sigma^* + 6{\cdot}447$	10	0·710	0·909	(14)
$\log\frac{1}{C} = 1{\cdot}113E_s^c + 3{\cdot}566\sigma^* - 4{\cdot}432n_H + 11{\cdot}911$	10	0·986	0·235	(15)
$\log\frac{1}{C} = 2{\cdot}486E_s^c - 0{\cdot}041\pi^2 + 0{\cdot}746\pi - 3{\cdot}516\sigma^* + 5{\cdot}740$	10	0·774	0·967	(16)
$\log\frac{1}{C} = 3{\cdot}744E_s^c + 0{\cdot}475\pi + 8{\cdot}799\sigma^{*2} - 8{\cdot}012\sigma^* + 6{\cdot}168$	10	0·910	0·633	(17)

E_s^c is the steric substituent constant corrected for hyperconjugation effects.[21] It is defined as $E_s^c = E_s + 0{\cdot}306\ (n - 3)$ where n is the number of hydrogens on the substituent which are hyperconjugated. For the methyl group, $n = 3$. We have set $n = 0$ for H. Better correlations were obtained with E_s^c than when E_s was used Addition of a π term to Eq. 15 does not result in an improved correlation. Equation 18 is derived from the noradrenaline antagonism of Table 2.

	n	r	s	
$\log\frac{1}{C} = 1{\cdot}123E_s^c + 3{\cdot}844\sigma^* - 4{\cdot}485n_H + 11{\cdot}858$	10	0·950	0·452	(18)

Not only are the coefficients in Eq. 18 very close to those of Eq. 15, equations analogous to 12–14, 16 and 17 also have very similar constants. The close mathematical agreement between Eq. 18 and Eq. 15 leaves no doubt about the similarity of the reaction mechanism antagonizing adrenaline and noradrenaline. The coefficients associated with n_H and σ^* in Eqs 15 and 18 are not at all close to the corresponding coefficients in Eq. 4 and hence indicate a difference between the dependence of base strength and the dependence of activity on the substituent constants.

Equation 19 comes from the data in Table 2 on adrenaline antagonism in mice.

	n	r	s	
$\log\frac{1}{C} = 0{\cdot}282E_s^c + 2{\cdot}703\sigma^* - 3{\cdot}034n_H$				
$+ 8{\cdot}954$	9	0·924	0·378	(19)

Comparing the intercept of Eq. 19 with those of Eqs 15 and 18, we see that the mouse protection test is a less sensitive test. This lack of sensitivity also shows up in the coefficients of E_s^c, n_H and σ^*. Although the signs are the same as in Eqs 15 and 18, the numerical values are smaller. The correlation in the mouse test is not as good as that of the cat test.

From the data in Table 3 se have obtained Eqs 20 and 21 for the antagonism of adrenaline and noradrenaline, respectively, on rat blood pressure by the 2-bromo-alkylamines.

	n	r	s	
$\log\frac{1}{C} = 0{\cdot}902E_s^c + 0{\cdot}830\sigma^* - 3{\cdot}082n_H$				
$+ 9{\cdot}858$	11	0·927	0·547	(20)
$\log\frac{1}{C} = 0{\cdot}811E_s^c + 0{\cdot}721\sigma^* - 2{\cdot}950n_H$				
$+ 9{\cdot}644$	11	0·952	0·420	(21)

The constants for the rat results are between those obtained with cats and with mice. However, in deriving these equations we did not employ examples 29 and 33, since these compounds were very poorly correlated. This is difficult to explain since the same compounds gave reasonable results in the cat and mouse tests.

Since poor results were obtained with both adrenaline and noradrenaline, it seems unlikely that it is due to a testing variation. It more likely reflects a metabolic difference in the test animals.

Equations 22 and 23 arise from the data in Table 4 on the antagonism of 5-HT by 2-bromoakylamines. Equation 22 pertains to work on rat uterus and Eq. 23 to that on guinea pig ileum.

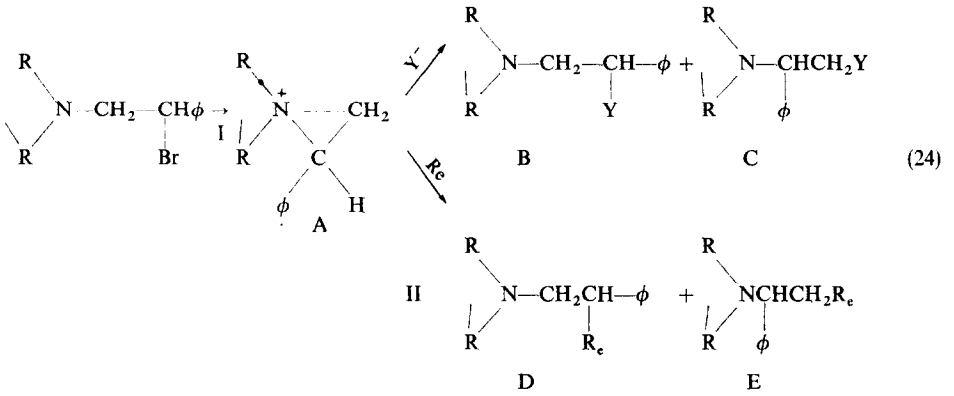
	n	r	s	
$\log\frac{1}{C} = 0{\cdot}038E_s^c + 4{\cdot}169\sigma^* - 3{\cdot}630n_H$				
$+ 7{\cdot}175$	8	0·985	0·174	(22)
$\log\frac{1}{C} = 0{\cdot}435E_s^c + 5{\cdot}249\sigma^* - 3{\cdot}532n_H$				
$+ 6{\cdot}090$	7	0·873	0·306	(23)

Of all the tests, the guinea pig ileum test is the least sensitive and gives the poorest correlation.

In all of the above examples we have explored all combinations of π, E_s^c and n_H. Since the results are very similar throughout, we have made a detailed report only for the first set of data (Eqs 12–17).

DISCUSSION

The critical steps, which from previous work must be considered in the mechanism of action of the haloalkylamines, are formulated in Eq. 24.

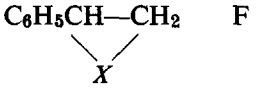


(24)

In Eq. 24, R_e represents a receptor site and Y^- represents any nucleophilic species other than R_e. In a careful analysis of the problem, Chapman and Triggle[30] point out that the formation of A, via step I, is extremely rapid and therefore step I can be excluded as being rate limiting. Since the immonium ion forms so rapidly, it is at once available for reaction at the receptor sites; however, this ion undergoes solvolysis with water present. Also, it may react with many other nucleophiles present in the blood or body tissue instead of R_e. As Chapman and Triggle point out, A may react either through an S_N1 or S_N2 mechanism with water or nucleophiles. In a study of model compounds, they investigated the solvolysis of a variety of immonium ions with a water–acetone mixture. From their results we derived Eqs 25 and 26.

	n	r	s	
$\log K = -0{\cdot}389E_s^c - 2{\cdot}034$	6	0·899	0·170	(25)
$\log K = -1{\cdot}100E_c^s + 4{\cdot}470\sigma^* - 1{\cdot}846$	6	0·998	0·031	(26)

In the above equations, K is the solvolysis rate at 31° in acetone–water (1:1). Substituent constants were available for six of the molecules studied. $X = N(CH_3)_2+$, NCH_3ET+, $N(Et)_2+$, $N(Pr)_2+$ $N(i\text{-}Pr)_2+$ and $N(Bu)_2+$ in formula F.



F

As in the equations correlating biological activity, we found E_s^c to give better correlations than E_s. It is quite interesting that E_s^c correlates the data so well, since the immonium ion system is rather different from the ester system from which E_s is derived. The quantitative conclusions of Eq. 26 are in line with the qualitative thinking of Chapman and Triggle. The use of regression analysis, however, enables one to assign relative importance to the substituent effects. It is evident from Eq. 25 that the steric effect of the groups on nitrogen is the most important driving force for solvolysis. The addition of the σ^* term in Eq. 26 is statistically quite significant ($F_{1,3} = 120$) even though it does not account for as much of the variance in the data as E_s^c. The

sign of the coefficient with E_s^c in Eq. 26 is opposite to that found in Eqs 12, 18, etc. That is, large groups promote the reaction of the immonium ion with water. Chapman and Triggle noted in qualitative terms that the order of reactivity in solvolysis did not parallel that of antiadrenaline activity. Comparison of Eq. 26 with Eqs 12, 18, etc. shows in quantitative terms that the big difference is due to the steric interactions. The inductive effects of the *N*-substituents as revealed by the coefficients associated with σ^* are in general much the same.

If we let A = haloamine, EI = ethyleneimmonium ion, R_e = receptor, and X = reaction product of R_e and EI, then at time t it is

$$\frac{[R_e] - [X]}{[R_e]}$$

that is being measured when a challenging dose of adrenaline is administered.

Since

$$\frac{[R_e] - [X]}{[R_e]} = 1 - \frac{[X]}{[R_e]}$$

and since $[A] \xrightarrow[\text{fast}]{\text{very}} [EI]$, whether Eqs 27 or 28 represents the correct mechanism $[X]$ would be a function of $[EI]$ assuming an S_N2 mechanism.

$$[R_e] + [EI] \rightarrow [R_e \cdot EI] \rightarrow [X] \tag{27}$$

$$[R_e] + [EI] \rightarrow [X] \tag{28}$$

To us, the results of Chapman and Triggle seem to favor an S_N2 mechanism for the most effective molecules for the reaction of EI and R_e. That is, for an S_N1 solvolysis, results embodied in Eqs 25 and 26 show a negative coefficient with E_s^c. For the results *in vivo* we find a positive coefficient with E_s^c. Although this does not prove the S_N2 mechanism, it does seem to us to be a more attractive working hypothesis. If an S_N2 mechanism does prevail, then the ratio $[X]/[R_e]$ would be a function of $[EI]$. One could also imagine the reaction occurring by an S_N1 mechanism provided that EI did not form the carbonium ion so readily that all of it reacted with water and nucleophiles in the blood before reaching R_e. $[X]/[R_e]$ would also be a function of $[EI]$ for an S_N1 mechanism. Since the results of Chapman and Triggle seem to favor an S_N2 mechanism for the reaction of EI and R_e, the ratio $[X]/[R_e]$ would be a function of $[EI]$. In our analysis we are assuming that the rate of formation of X is a function of E_s^c, σ^* and n_H. Since the hydrophobic character of the substituent on the benzene ring (π) does play an important role (Eqs 7 and 11), it seems to us that Eq. 27 is the most likely mechanism of action. For groups on nitrogen, π does not have a *discernible* role. However, because of covariance in E_s^c, σ^* and π this might be masked by the combined constants E_s^c and σ^*. The study of a better selection of substituents could clarify this point. If π of the substituents on nitrogen can be neglected, as the data in hand indicate, then this could be interpreted to mean that the drug is bound to the receptor sites hydrophobically via the ring. Alkylation of a nucleophile then occurs. We tend to think of this order of events rather than the reverse because the nonspecific nature of hydrophobic bonding would make this reaction more rapid than highly specific S_N2 attack of the nucleophile on the ethylene immonium ion. In fact, the role of the phenyl group and its substituents may be one of orienting the immonium

ion for ease of nucleophilic alkylation. The poor fit of compound 23 indicates that the hydrophobic region is of rather limited size.

If then we can consider the over-all reaction to be rate limited by an S_N2 step according to Eq. 27 and the primary function of the ring substituents to aid in hydrophobic bonding, what is the role of nitrogen substituents delineated by Eqs 12 and 19–23? Considering first the role of E_s^c, it is opposite to that of solvolysis shown in Eq. 26. Large groups promote S_N1 solvolysis; large groups lower adrenaline antagonizing ability. The obvious explanation for this is that large groups on nitrogen, through steric repulsion with the phenyl group, weaken the C—N bond and increase the probability that the ion will react with water or other nucleophiles before it reaches the receptors. It is interesting to consider the size of the constant associated with E_s^c for each of the different tests. The larger the animal, the greater the dependence of activity on E_s^c. The order is cat > rat > mouse > uterus. In the case of the uterus, we find a very small constant. This order makes sense in terms of the resistance of the ion to solvolysis and reaction with nucleophiles before reaching the receptors. In the larger animals a longer time will be required for circulation through the more extensive capillary bed and hence a greater opportunity for side reaction is present. Side reactions are at a minimum in the case of direct application to the uterus. This is, of course, an oversimplified view since the approachability and reactivity of the receptors is also involved. The more active the receptors, the better able they are to compete with other nucleophiles for the immonium ions.

The roles of the constants σ^* and n_H are both opposite to those in Eq. 4; that is, when both σ^* and n_H operate to lower the base strength of the alkylamine, a stronger blocking agent results. Care must be taken in considering the role of n_H in these equations to be simply that of an effect on base strength. We are dealing with two sets of compounds, one having a hydrogen atom on nitrogen and one lacking such an atom. The role of n_H is simply to move two parallel curves together and it may be more complex than we have suggested above. It would be most interesting to include primary amines in the study to see if the single parameter n_H would allow treatment of all three classes of bases in one equation.

If the critical step is truly like Eq. 27, then the positive coefficient with σ^* and the negative coefficient with n_H do make sense. Base strengthening groups would not favor an S_N2 mechanism.

It is gratifying that the biological activity of such a diverse group of 2-haloalkylamines can be quantitatively correlated by using substituent constants. Our results suggest that more active derivatives could be obtained by using more lipophilic groups on the ring, provided that they are not too electron-withdrawing. It also seems that further deactivation of the ion with respect to S_N1 type activity up to an as yet undetermined point should yield more selective antagonists. As discussed above, this would depend on the type of animal or tissue used in testing. Little is to be gained from modifying the size of the functions on nitrogen with very small animals or tissue. Small electron-withdrawing groups on nitrogen would, in general, be most suitable. Whether a unit such as $\begin{matrix}CF_3\\CH_3\end{matrix}\!\!>\!\!N$ would do it is difficult to say. The electron-withdrawing power of CF_3 would be good; however, this might be offset by its larger size. Groups such as $F_2C{=}CH{-}$ or $HC{\equiv}C{-}$ might be quite advantageous sterically.

How much the *pi* electrons would tend to neutralize the positive charge on nitrogen is difficult to say, since the necessary substituent constants are not available for the calculations. Possibly, more selectivity for the S_N2 reaction could be built in by decreasing the electron density on the 1-carbon by using fluorine: $(CH_3)_2NCHBrCF_2\phi$.

The approach we have used here in the analysis of the adrenergic blockers would be interesting to apply in the development of anticancer nitrogen mustards.

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