Br. J. Pharmac. (1969), **37**, 555–584.

The affinity and activity of compounds related to nicotine on the rectus abdominis muscle of the frog (*Rana pipiens*)

R. B. BARLOW AND G. M. THOMPSON (AND, IN PART, N. C. SCOTT)

Department of Pharmacology, University of Edinburgh

1. Series of pyridylalkyl- and substituted phenylalkyl-trimethylammonium salts, triethylammonium salts, diethylamines and di-*n*-propylamines have been made. The substituents in the benzene ring were nitro, chloro, bromo, methoxy, hydroxy and amino groups and the alkyl residues had one, two, or three methylene groups separating the aromatic nucleus from the cationic head.

2. Most of the trimethylammonium compounds caused a contracture of the frog rectus muscle, but some were partial agonists and a few were antagonists. The di-*n*-propylamines were all antagonists, as were most of the diethylamines and triethylammonium compounds, though some of these were partial agonists and a few triethylammonium compounds were agonists. The affinities of the antagonists and partial agonists for the receptors stimulated by β-pyridylmethyltrimethylammonium (and by nicotine) were measured. The equipotent molar ratios of all the agonists were measured relative to β-pyridylmethyltrimethylammonium.

3. The dissociation constants of the pyridylmethyldiethylamines and substituted benzyldiethylamines were measured. The effects of substituents on the pK_a of benzyldiethylamine were similar to their effects on the pK_a of aniline, though there were differences with some of the *o*-substituted compounds, which could be attributed to internal hydrogen-bond formation.

4. There is no obvious correlation between the effects of a substituent on the pK_a of benzyldiethylamine and its effects on affinity. Although increasing the size of the cationic group usually increased affinity, it did not always do so. The compounds with the highest affinity, *p*-hydroxybenzyldiethylamine (log K, 5·90) had about half the affinity of (+)-tubocurarine (log K, 6·11), but the triethylammonium analogue (log K, 4·17) had only about one-fiftieth of the affinity of the tertiary base. The binding of the drug to the receptor appears to involve many factors which include the size of the groups as well as their electron-releasing or withdrawing nature and other properties, such as their polar and lipophilic or lipophobic character.

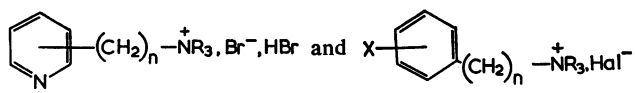
5. There is no obvious correlation between the effects of a substituent on the affinity of the diethylamino or triethylammonium compounds and its effects on the activity of the trimethylammonium analogue. The most active compounds contain hydroxy- and amino-, phenyl or β-pyridyl groups, *m*-hydroxyphenylpropyltrimethylammonium being about 50 times as active as nicotine, but the

corresponding diethylamino or triethylammonium compounds do not have high affinity. There does not seem necessarily to be an inverse relationship between activity and affinity, however, because some *m*-nitro and *m*-chloro trimethylammonium compounds have considerable activity and the analogous triethylammonium compounds have considerable affinity.

6. It is suggested that ability to activate these receptors is associated with the presence of substituents which can interact with water molecules which may be involved in the action of the drug at the receptor.

Ever since the work of Hey (1952), there have been many attempts to correlate chemical structure with nicotine-like activity (Ormerod, 1956; Sekul & Holland, 1961a, b; Barlow & Hamilton, 1962; Wong & Long, 1962; Haglid, 1965, 1967; Coleman, Hume & Holland, 1965; Kier, 1968). These have all been unsatisfactory because they have been unable to assess how far changes in chemical structure alter the ability of a molecule to fit the nicotine-sensitive receptors (its affinity) and how far they alter its ability to activate them (its efficacy). A particular change in structure may increase activity because it has increased affinity, without affecting efficacy, whereas a different change in structure may produce exactly the same increase in activity by increasing efficacy, without altering affinity.

To obtain some idea of the effects of changes in structure on affinity, we have therefore prepared series of compounds, which are antagonists or only partial agonists at the nicotine-sensitive receptors of the frog rectus abdominis muscle, and measured their affinity by methods described by Barlow, Scott & Stephenson (1967). We have also compared the relative activity of analogous series of agonists on the preparation to see how far it may be possible to assess the effects of changes in chemical structure on efficacy. The compounds had the structure:



where n was 1, 2 and 3, X was NO_2, Cl, Br, MeO, HO, H_2N, and $\overset{+}{N}R_3$ was $\overset{+}{N}Et_2H$, $\overset{+}{N}Et_3$, $\overset{+}{N}nPr_2H$ and $\overset{+}{N}Me_3$; they are listed in Table 8. In most series, the compounds with $\overset{+}{N}Me_3$ groups were agonists and the others partial agonists or antagonists.

The effect of the phenyl substituent, X, on electron distribution is usually supposed to be important for nicotine-like activity. With these particular compounds it should range from marked release of electrons (NH_2) to marked withdrawal (NO_2). In the pyridyl compounds, the insertion of nitrogen in place of the $-CH=$ of the benzene ring should have effects on activation similar to the substitution of hydrogen by a nitro group. We have attempted to assess the effects of changes in chemical structure on electron distribution by comparing the pK_a values of substituted benzyldiethylamines and pyridylmethyldiethylamines with those of benzyldiethylamine itself. The observed changes in pK_a have then been compared with the changes in affinity and in activity.

Methods

Frog rectus preparation

The rectus abdominis muscle from *Rana pipiens* was used in all experiments. The method of setting up the preparation and using it to measure the affinity constants of the antagonists and partial agonists and the relative activity of agonists was the same as that described in full by Barlow *et al.* (1967). It was possible to use the same interval (30 min) between doses of agonist as was used with the simple onium salts previously studied.

Antagonists

Estimates of the logarithm of the affinity constant were made on several preparations, usually about five, and are expressed as the mean value ± the standard error. In most experiments, β-pyridylmethyltrimethylammonium was used as agonist, but, in experiments with pyridylmethyl compounds and with some of the pyridylethyl compounds, tetramethylammonium and, occasionally, carbachol were used as agonists. The choice of agonist did not appear to affect the result, though we have not made systematic experiments to check this.

Although it was hoped that chemically similar series of compounds would be acting competitively, it was found that many of the compounds were not acting competitively in high concentrations. The measurements of affinity were therefore made only with concentrations of antagonist which produced small dose-ratios, less than 10 and usually around 5. The results of tests for competition with series of antagonists on the guinea-pig ileum by Abramson, Barlow, Mustafa & Stephenson (1969) show a similar incidence of non-competitive antagonism with high concentrations of certain compounds, which nevertheless appeared to be acting competitively in lower concentrations. With some of the compounds used in the present work, however, the non-competitive component appears to be bigger than in the compounds tested on the guinea-pig ileum, hence the need to use even lower dose-ratios.

Partial agonists

Estimates of the logarithm of the affinity constant were made on several preparations, usually about five, and are expressed as the mean value ± the standard error. Both the addition method (Stephenson, 1956) and the reciprocal plot method (Barlow *et al.*, 1967) were used to obtain results. Barlow *et al.* (1967) have shown that estimates obtained by these methods do not differ significantly ; we have found it convenient to use the first method for partial agonists which produce only small responses, and the second for those which produce responses which are more than half the size of the maximum response to a pure agonist. Some of the compounds had such low efficacy that it was possible to test them in concentrations at which they behaved as antagonists.

Agonists

Estimates of the relative activity of the agonists were made on several preparations, usually about five, and are expressed as the mean value of the logarithm of the equipotent molar ratio relative to β-pyridylmethyltrimethylammonium, ± the standard error. This compound was chosen as standard because it was known to be

highly active (Barlow & Hamilton, 1962), was easy to prepare, and was easily washed out of the tissues, being a quaternary ammonium salt.

The pK_a *values* were determined by the method of Albert & Serjeant (1962), with a 10^{-4} mole sample of the hydrobromide, or dihydrobromide, of the base, dissolved in about 40 ml. of a mixture of ethanol and water (40% by volume). The solution was maintained at 25° ±0·2° C and stirred by a steady stream of nitrogen. It was titrated against 0·05 N NaOH using a Pye Dynacap pH meter fitted with glass and calomel electrodes. It was necessary to use ethanol because many of the bases did not dissolve adequately in water. The pK_a values are depressed as a result. The value for benzyldiethylamine is the mean of twelve experiments and the standard error of the mean is 0·03. The results for all the other compounds are the mean of two determinations, which did not differ by more than 0·02 of a unit.

Results

Estimates of pK_a

The results of the pK_a measurements are shown in Table 1A. From these, the differences between the pK_a of the substituted and unsubstituted compounds have been calculated and are shown in Table 1B. These differences have been taken as a measure of the effects of substituents on the electron distribution.

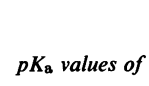
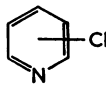
Although the biological results for the tertiary bases should all be corrected for the degree of ionization, the pK_a values indicate that the compounds are all largely present as the ion. The weakest base was γ-pyridylmethyldiethylamine, which had a pK_a of 7·69 in the ethanol-water mixture (40% ethanol v/v). In pure water, however, the pK_a was 8·18 at 25° C, and at room temperature, at which the biological tests were performed, the value will be higher still. The degree of ionization is likely to exceed 75%. The corrections which should be made to the estimates of the log. affinity constants or log. equipotent molar ratios will not exceed 0·125, and we have, therefore, not thought it necessary to produce separate tables of results for the tertiary bases, calculated in terms of ionic concentrations.

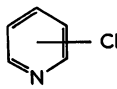
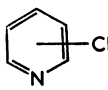
Biological results

The results of the tests on the frog rectus preparation are shown in Tables 2, 3 and 4. The compounds which are pure agonists are indicated by bold type and the values indicate the logarithm of the equipotent molar ratio relative to β-pyridylmethyltrimethylammonium. Partial agonists are indicated by italics and the values, like those for the antagonists, are the logarithm of the affinity constant. An indication of the efficacy of the partial agonists is given by the method used for estimating their affinity. In the tables, A indicates that the reciprocal plot method was used (see **Methods**). B indicates that the addition method was used and C that the compounds had very low efficacy indeed and were treated as antagonists. Values for phenylpropyltriethylammonium were obtained both by the reciprocal plot method and by the addition method. The results, 4·24 (A) and 3·84 and 4·00 (B), suggest that slightly higher values may be obtained with the reciprocal plot method, but, as was observed in previous comparisons of the two methods (Barlow *et al.*, 1967), the differences are small and may not be significant.

For convenience the compounds with the highest affinity and those with the highest activity are collected in Table 5. Apart from the compounds with very long

TABLE 1

A. *pK_a values of the tertiary bases*  *and*  *in an ethanol–water mixture* (40% *v/v ethanol*) *at* 25° *C*

X=	$(CH_2)_nNR_2$=	*o*	*m*	*p*
H	CH_2NEt_2		8·90 ±0·03(12)	
NO_2		8·05	7·96	7·81
Cl		8·37	8·29	8·43
Br		—	8·27	8·43
MeO		9·49	8·58	8·83
HO		8·31	8·56	8·99
NH_2		8·70	9·12	9·53
		*α* 8·39 8·74*	*β* 8·13 8·55*	*γ* 7·69 8·18*
		8·77*	8·58*	8·16*

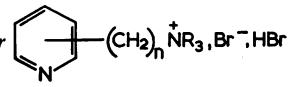
		o	*m*	*p*
H	$(CH_2)_2NEt_2$		9·14	
Cl	$(CH_2)_2NEt_2$	8·71	8·91	Not measured
Cl	$(CH_2)_3NEt_2$	9·31	9·46	9·50
H	CH_2NMe_2		8·07	
Cl		7·88	7·75	7·94
NH_2		8·39	8·58	8·94
H	$(CH_2)_2NMe_2$		8·63	
Cl	$(CH_2)_2NMe_2$	8·33	8·26	Not measured
Cl	$(CH_2)_3NMe_2$	8·75	8·59	8·79

The values marked with an asterisk were obtained in pure water. The pK_a of benzyldiethylamine is the mean of twelve estimates ± the standard error; other values are the means of two estimates which did not differ by more than 0·02.

B. *Effects of substituents on the pK_a of benzyldiethylamine*

Substituent	$\triangle pK_a$
γ-pyridyl	−1·23
p-NO_2	−1·11
m-NO_2	−0·96
o-NO_2	−0·87
β-pyridyl	−0·79
m-Br	−0·65
m-Cl	−0·63
o-OH	−0·61
o-Cl	−0·55
α-pyridyl	−0·53
p-Br	−0·49
p-Cl	−0·49
m-OH	−0·36
m-OMe	−0·34
o-NH_2	−0·22
p-OMe	−0·09
p-OH	+0·07
m-NH_2	+0·20
o-OMe	+0·57
p-NH_2	+0·61

$\triangle pK_a = pK_a$ compound $- pK_a$ benzyldiethylamine; positive values indicate that the substituent is base-strengthening (electron-releasing); negative values indicate that it is base-weakening (electron-withdrawing).

TABLE 2. *Results for* 

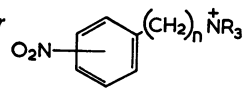
n=1		α	β	γ
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	*3·05* (A) ±0·05 (4)	**0·0** (standard)	**1·35** ±0·05 (4)
	$\overset{+}{N}Me_2Et$	—	**1·44** ±0·07 (6)	—
	$\overset{+}{N}MeEt_2$	—	*3·62* (A) ±0·10 (5)	—
	$\overset{+}{N}Et_2H$	3·02 ±0·05 (4)	*2·61* (B) ±0·15 (4)	3·27 ±0·01 (8)
	$\overset{+}{N}nPr_2H$	3·74 ±0·04 (6)	3·72 ±0·08 (8)	4·14 ±0·11 (6)
	$\overset{+}{N}nBu_2H$	—	4·36 ±0·11 (4)	—
	$\overset{+}{N}Et_3$	3·41 ±0·10 (7)	3·68 ±0·10 (6)	3·85 ±0·08 (5)
n=2	$\overset{+}{N}Me_3$	**2·45** ±0·03 (6)	**−0·23** ±0·07 (5)	*3·23, 3·26**(B)
	$\overset{+}{N}Et_2H$	3·40 ±0·05 (4)	*3·08* (C) ±0·11 (4)	3·33 ±0·09 (3)
	$\overset{+}{N}nPr_2H$	4·13 ±0·09 (9)	3·39 ±0·06 (7)	3·95 ±0·05 (3)
n=3	$\overset{+}{N}Me_3$	—	**1·08** ±0·08 (5)	**0·69** ±0·04 (5)
	$\overset{+}{N}Me_2H$	—	*3·41* (A) ±0·13 (6)	
	$\overset{+}{N}Et_2H$	—	2·98 ±0·06 (6)	2·77 ±0·04 (5)
	$\overset{+}{N}nPr_2H$	—	3·52 ±0·07 (4)	3·66 ±0·03 (8)

(−)Nicotine	**0·99**±0·04 (4)
(−)Nicotine monomethiodide	**0·72**±0·01 (3)
(−)Anabasine	**1·19**±0·02 (5)
(+)Tubocurarine	6·16±0·01 (4)
Choline phenylether	**0·89**±0·03 (4)

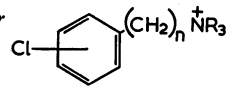
The numbers show log K for the antagonists and for the partial agonists, which are indicated by italics. Numbers in bold type show the log e.p.m.r. of agonists relative to β-pyridylmethyltrimethylammonium. (See page 559.)

The asterisk indicates that only two results were obtained with a sample of this material, which was unstable and impure. (See page 581.)

TABLE 3

Results for 

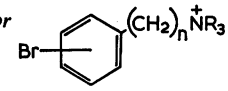
n=1		*o*	*m*	*p*
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	*3·32* (B) ±0·04 (6)	**1·88** ±0·05 (5)	**1·67** ±0·02 (5)
	$\overset{+}{N}Et_2H$	4·41 ±0·05 (6)	*2·46* (A) ±0·03 (5)	*2·27* (A) ±0·07 (5)
	$\overset{+}{N}Et_3$	—	4·33 ±0·04 (7)	3·11 ±0·14 (6)
n=2				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**1·71** ±0·02 (4)	**0·62** ±0·01 (4)	**1·66** ±0·03 (7)
	$\overset{+}{N}Et_3$	4·41 ±0·04 (5)	5·30 ±0·07 (6)	**3·00** ±0·02 (5)

Results for 

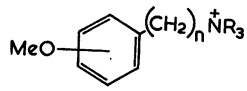
n=1		*o*	*m*	*p*
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	*3·41* (B) ±0·05 (4)	**1·71** ±0·03 (6)	**1·80** ±0·02 (6)
	$\overset{+}{N}Et_2H$	4·65 ±0·07 (6)	4·69 ±0·04 (3)	5·37 ±0·15 (7)
n=2				
$\overset{+}{N}R_3$	$\overset{+}{N}Me_3$	**2·26** ±0·03 (5)	**0·70** ±0·02 (6)	**1·40** ±0·03 (6)
	$\overset{+}{N}Et_3$	4·77 ±0·02 (4)	*4·00* (A) ±0·13 (5)	**1·48** ±0·10 (8)
n=3				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**1·01** ±0·03 (4)	**2·57** ±0·03 (7)	*3·85* (A) ±0·04 (5)
	$\overset{+}{N}Et_3$	*5·29* (C) ±0·05 (5)	*5·15* (C) ±0·07 (4)	*5·02* (C) ±0·07 (5)

The numbers show log K for the antagonists and for the partial agonists, which are indicated by italics. Numbers in bold type show the log e.p.m.r. of agonists relative to β-pyridylmethyltrimethylammonium. (See page 559.)

Table 3 (continued)

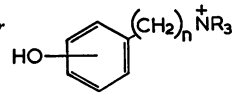
Results for 

n=1		*o*	*m*	*p*
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	—	**1·12** ±0·03 (4)	**1·64** ±0·07 (4)
	$\overset{+}{N}Et_2H$	—	*4·56* (C) ±0·09 (4)	*4·05* (C) ±0·10 (4)
	$\overset{+}{N}Et_3$	—	*4·67* (C) ±0·07 (6)	4·49 ±0·02 (5)
n=2				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	4·20 ±0·02 (4)	**1·90** ±0·06 (4)	**1·85** ±0·03 (4)

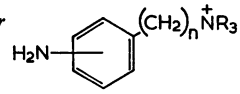
Results for 

n=1		*o*	*m*	*p*
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	*2·90* (B) ±0·17 (4)	**2·89** ±0·03 (3)	**1·62** ±0·05 (4)
	$\overset{+}{N}Et_2H$	*2·14* (A) ±0·11 (5)	*2·57* (A) ±0·09 (4)	*2·73* (A) ±0·06 (6)
n=2				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**2·57** ±0·08 (4)	**2·22** ±0·04 (5)	*3·13* (B) ±0·14 (6)
	$\overset{+}{N}Et_3$	4·44 ±0·05 (5)	*4·42* (C) ±0·03 (4)	*3·76* (A) ±0·06 (5)
n=3				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**1·32** ±0·05 (3)	**1·96** ±0·02 (4)	**2·80** ±0·09 (4)
	$\overset{+}{N}Et_3$	4·32 ±0·06 (6)	*3·66* (A) ±0·10 (6)	*4·51* (C) ±0·03 (6)

Table 3 (continued)

Results for 

		o	*m*	*p*
n=1				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**2·40** ±0·04 (6)	**1·67** ±0·04 (6)	**2·37** ±0·01 (5)
	$\overset{+}{N}Et_2H$	3·54 ±0·06 (4)	*3·31* (A) ±0·13 (5)	5·90 ±0·03 (8)
	$\overset{+}{N}Et_3$	—	3·96 ±0·03 (4)	4·17 ±0·02 (4)
n=2				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**2·42** ±0·02 (4)	**−0·25** ±0·04 (4)	**0·75** ±0·02 (4)
	$\overset{+}{N}Et_3$	4·27 ±0·07 (6)	**2·24** ±0·08 (4)	**2·39** ±0·03 (4)
n=3				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**1·63** ±0·03 (4)	**−0·70** ±0·03 (4)	**1·30** ±0·06 (4)
	$\overset{+}{N}Et_3$	4·43 ±0·03 (6)	*3·72* (A) ±0·07 (6)	*3·58* (A) ±0·13 (3)

Results for 

		o	*m*	*p*
n=1				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	4·05 ±0·13 (3)	**2·71** ±0·04 (6)	*3·13*(A) ±0·08 (5)
	$\overset{+}{N}Et_2H$	3·93 ±0·08 (6)	3·47 ±0·02 (6)	*2·84* (B) ±0·16 (5)
n=2				
$\overset{+}{N}R_3$=	$\overset{+}{N}Me_3$	**0·56** ±0·13 (5)	**0·26** ±0·02 (4)	**−0·42** ±0·01 (5)
	$\overset{+}{N}Et_3$	*4·08* (B) ±0·05 (4)	*3·91* (B) ±0·10 (6)	*4·42* (B) ±0·06 (4)

The numbers show log K for the antagonists and for the partial agonists, which are indicated by italics. Numbers in bold type show the log e.p.m.r. of agonists relative to β-pyridylmethyltrimethylammonium. (See page 559.)

chains, the tertiary base, *p*-hydroxybenzyldiethylamine had the highest affinity, about one quarter of that of (+)-tubocurarine. The corresponding triethylammonium salt had much lower affinity but *p*-chlorobenzyldiethylamine had about half the affinity of the *p*-hydroxy compound. The *o*-, *m*-, and *p*-chlorophenylpropyltriethylammonium salts were remarkable in that they combined high affinity with some degree of efficacy. Most partial agonists were found to have low affinity, particularly those which had sufficient efficacy to be tested by the reciprocal plot or addition methods. The chlorophenylpropyltriethylammonium salts, however, all had such low efficacy that their affinity was estimated by testing them in concentrations in which they behaved as antagonists.

Many of the agonists are extremely potent. The standard, β-pyridylmethyltrimethylammonium, is about 10 times as active as nicotine and the most potent compound, *m*-hydroxyphenylpropyltrimethylammonium, is about 50 times as active as nicotine. Our results for *m*-hydroxyphenethyltrimethylammonium (leptodactyline) indicate that it is just under 20 times as active as nicotine, whereas the results

TABLE 4. *Results for* $Ph-(CH_2)_n-\overset{+}{N}R_3$

	$\overset{+}{N}Et_2H$	$\overset{+}{N}Et_3$	$\overset{+}{N}nPr_2H$	$\overset{+}{N}Me_3$
n=1	*4·15* (C)	4·03	4·59	**1·97**
	±0·09 (6)	±0·05 (7)	±0·13 (5)	±0·04 (6)
2	*4·17* (C)	*3·39* (A)	4·18	**1·05**
	±0·07 (5)	±0·16 (4)	±0·10 (6)	±0·01 (4)
3	4·48	*4·03* (A,B)	—	**0·58**
	±0·05 (6)	±0·10 (3)		±0·03 (4)
4	—	5·08	—	**1·61**
		±0·04 (4)		±0·03 (4)
5	—	5·10	—	**1·23**
		±0·05 (5)		±0·02 (8)
6	—	6·30	—	*6·14* (C)
		±0·05 (6)		±0·06 (6)
7	—	6·52	—	6·03
		±0·05 (6)		±0·05 (4)

The significance of the numbers is indicated at the end of Table 3.

TABLE 5A. *Compounds with high affinity*

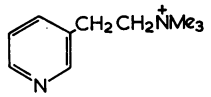
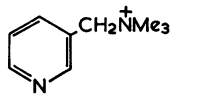
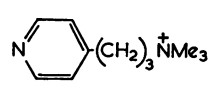
Compound	log K
(+)-Tubocurarine	6·16
p-$HOC_6H_4CH_2NEt_2$	5·90
p-$ClC_6H_4CH_2NEt_2$	5·37
m-$O_2NC_6H_4(CH_2)_2\overset{+}{N}Et_3$	5·30
o-$ClC_6H_4(CH_2)_3\overset{+}{N}Et_3$	*5·29* (C)
m-$ClC_6H_4(CH_2)_3\overset{+}{N}Et_3$	*5·19* (C)
p-$ClC_6H_4(CH_2)_3\overset{+}{N}Et_3$	*5·02* (C)
o-$ClC_6H_4(CH_2)_2\overset{+}{N}Et_3$	4·77
m-$ClC_6H_4CH_2NEt_2$	4·69
m-$BrC_6H_4CH_2\overset{+}{N}Et_3$	*4·67* (C)
o-$ClC_6H_4CH_2NEt_2$	4·65

Values in italic figures are log affinity constants of partial agonists and the letter indicates the method used to measure them.

of Erspamer & Glässer (1960) indicate that it is between 50 and 100 times as active. The difference may be due to differences in experimental technique and to the species of frog used. Erspamer & Glässer do not specify the species and it seems likely to have been *Rana esculenta*. We have performed a few experiments with these compounds using *Rana temporaria* and found that the results are very different from those obtained with *Rana pipiens*.

It is very striking that the most active agonists are quaternary salts. None of our tertiary bases was particularly active. The most active non-quaternary compound appears to be cytisine, which was tested on this preparation by Barlow & McLeod (1969), whose result is included in Table 5.

TABLE 5B. *Compounds with high activity*

Compound	log e.p.m.r.
m-$HOC_6H_4(CH_2)_3\overset{+}{N}Me_3$	−0·70
p-$H_2NC_6H_4(CH_2)_2\overset{+}{N}Me_3$	−0·42
m-$HOC_6H_4(CH_2)_2\overset{+}{N}Me_3$	−0·25
	−0·23
	Standard
m-$H_2NC_6H_4(CH_2)_2\overset{+}{N}Me_3$	+0·26
o-$H_2NC_6H_4(CH_2)_2\overset{+}{N}Me_3$	0·56
$C_6H_5(CH_2)_3\overset{+}{N}Me_3$	0·58
m-$O_2NC_6H_4(CH_2)_2\overset{+}{N}Me_3$	0·62
	0·69
(−)cytisine	0·70
m-$ClC_6H_4(CH_2)_2\overset{+}{N}Me_3$	0·70
(−)nicotine monomethiodide	0·72
p-$HOC_6H_4(CH_2)_2\overset{+}{N}Me_3$	0·75
Cholinephenylether	0·89
(−)nicotine	0·99
o-$ClC_6H_4(CH_2)_3\overset{+}{N}Me_3$	1·01
$C_6H_5(CH_2)_2\overset{+}{N}Me_3$	1·05
m-$BrC_6H_4CH_2\overset{+}{N}Me_3$	1·12
(−)Anabasine	1·19
p-$HOC_6H_4(CH_2)_3\overset{+}{N}Me_3$	1·30

The value for cytisine was obtained by Barlow & McLeod (1969).

Discussion

Relationships between chemical structure and pK_a

The effect of a group on electron distribution can be assessed by its effect on the pK_a of an acid or base. For aromatic compounds it is most common to use the change in the pK_a of benzoic acid. Hammett (1937) suggested that the rate constants and equilibrium constants for many reactions empirically fitted the equation $\log K/K_o = \sigma\rho$, where K was the constant for the substituted compound, K_o that of the parent compound, σ a constant for the substituent group and ρ a constant for the reaction, solvent and temperature. He took the dissociation of substituted benzoic acids as a standard for comparison, so the equation becomes, $pK_o - pK = \sigma$ ($\rho = 1$).

Tables of values of σ have been compiled by many workers (Clark & Perrin, 1964; Albert, 1968; Wells, 1968) and Clark & Perrin have shown that it is possible to calculate the pK_a of substituted anilines from the equation $pK_a = 4{\cdot}57 - 2{\cdot}81\sigma$; 4·57 being the pK_a of aniline in water at 25° C and 2·81 the value for ρ calculated from the measurement of the pK_a values of a number of substituted anilines. Values for *m*-substituted compounds fit this equation satisfactorily, but some values for *p*-substituted compounds, notably for *p*-nitro, lie off the line because the resonance effects of the group are different in the two series of compounds. Values for *o*-substituents are particularly unreliable. In addition to the differences between resonance effects in different series of compounds, *o*-substituents produce steric effects, both by reason of their bulk and, in some instances, because internal hydrogen bonding stabilizes one particular form of the compound. These steric effects will be quite different in the anilines, for example, from what they are in the benzoic acids.

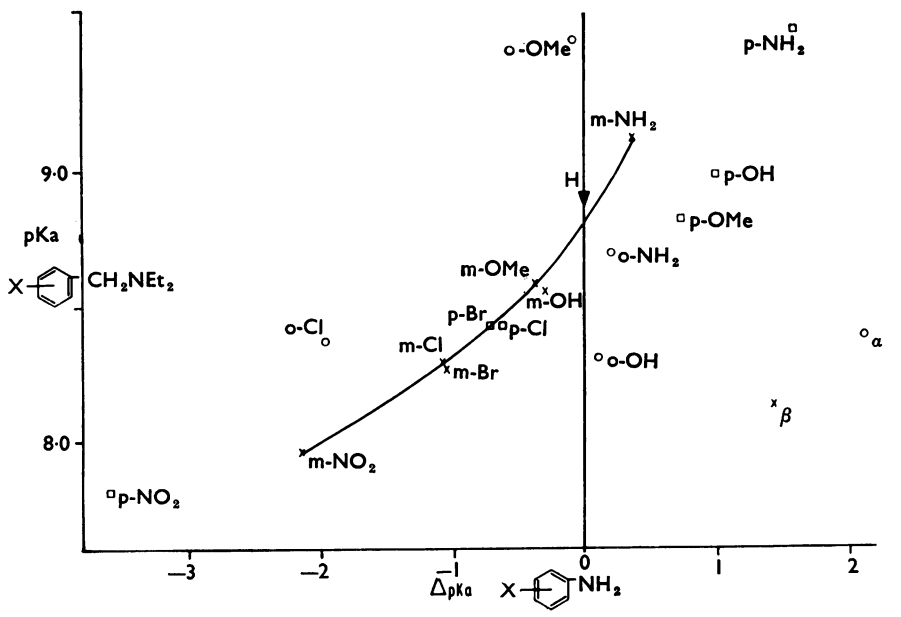


FIG. 1. Effects of substituents on pK_a: ordinate, pK_a of substituted benzyldiethylamine in 40% ethanol; abscissa, effect of substituent on pK_a of aniline, expressed as pK_a aniline $- pK_a$ substituted aniline. The line joins the values for *m*-substituted compounds; α and β refer to the pyridylmethyldiethylamines.

Although, like Hey (1952), Ormerod (1956) and later workers, we could have used σ values as an indication of the effects of substituents on electron distribution, it seemed better to use values derived from benzyldiethylamines for three reasons. First, the resonance effects of substituents should be much smaller in the compounds where the basic group is separated by methylene from the aromatic ring. Second, the effects of *o*-substituents are likely to be different in compounds with the extra methylene group. Third, intramolecular hydrogen bonding between *o*-substituents and the basic group will be different if this is tertiary rather than primary.

Figure 1 shows the relationship between our values of the pK_a of the benzyldiethylamines, dissolved in a mixture of ethanol and water (40% ethanol by volume), and the effects of substituents on the pK_a of the corresponding anilines. All values are for measurements at 25° C and the results for the anilines were taken from the tables compiled by Perrin (1965). It should be noted that positive values of σ indicate that the pK_a of the compound is less than that of benzoic acid or aniline, so the substituent is acid-strengthening or base weakening, that is, electron-withdrawing. The values of $\triangle pK_a$ shown in Fig. 1 are positive if the substituent is base-strengthening and electron-releasing. The results for the *m*-compounds appear to fit a curve rather than a straight line. This departure from the Hammett equation is not unusual (Wells, 1963) and possibly occurs because we used ethanol and water as a solvent. The effect of the change of solvent would be different in compounds containing groups which are hydrated from what it is in compounds containing groups which are not hydrated. The results for the *p*-compounds lie off the line and indicate that resonance effects are much more important in the anilines than in the benzyldiethylamines. This could account also for the high basicity of *o*-chlorobenzyldiethylamine. The low basicity of *o*-hydroxybenzyldiethylamine is likely to be due to stabilization of the unionized form by internal hydrogen bonding (Fig. 2A). This may occur to a lesser extent in the *o*-amino compound. The high basicity of the *o*-methoxy compound is likely to be due to stabilization of the ionized form (Fig. 2B).

Figure 1 also shows values for α- and β-pyridylmethyldiethylamines, for which $\triangle pK_a$ is the difference between the pK_a of the aminopyridine and that of aniline. In α-aminopyridine the basicity is greatly enhanced by resonance stabilization of the ionized form, which occurs to an even greater extent in γ-aminopyridine, which has a pK_a of 9·17. This cannot occur in the compounds with the extra methylene group. It should also not occur in β-aminopyridine, and the fact that the result for this compound lies well off the curve is difficult to explain. It seems likely, however, that it is the pK_a of β-aminopyridine which is high, rather than our value for β-pyridylmethyldiethylamine which is too low, because the dipole moment of pyridine, and its reactivity with nucleophilic reagents such as NH_2^-, suggests that the nitrogen in the ring is electron-withdrawing and in many ways comparable with a nitro group.

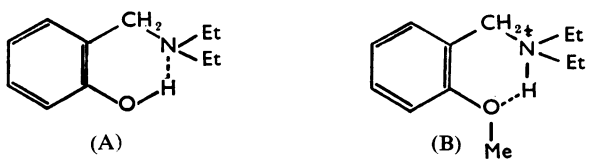


FIG. 2. A: Stabilization of the unionized form of *o*-hydroxybenzyldiethylamine by internal hydrogen-bonding. B: Stabilization of the ionized form of *o*-methoxybenzyldiethylamine by internal hydrogen-bonding.

From Fig. 1 it can be seen that it makes some difference whether the effects of a group on electron distribution are assessed from changes in the pK_a of the benzyldiethylamines or from the anilines (or from σ values for the benzoic acids which can be related to effects on the pK_a of the anilines). The differences are quantitative, rather than qualitative, however, except with *o*-substituents. It is important to establish that it is possible to correlate changes in structure with changes in basicity because, if this cannot be done, it is clearly unlikely that there can be any real understanding of the relationships between chemical structure and biological activity.

In addition to using the values of $\triangle pK_a$ (pK_a of the compound – pK_a of benzyldiethylamine) for the benzyldiethylamines as an indication of the effect of the substituent on electron distribution in the compounds with one methylene group in the side-chain, we have used the same values for the other series of compounds with two and three methylene groups. This has been necessary because we did not usually make the tertiary bases in these latter series. Although the extra methylene groups should make little difference to the effects of *m*- and *p*-substituents, they may make a considerable difference to the effects of *o*-substituents. Internal hydrogen bonding, such as shown in Fig. 2A and B, for instance, would be unlikely. Further, hydrogen bonding involving a proton on the basic group, as in Fig. 2B, would be impossible with quaternary salts, and this will apply to the compounds with only one methylene group which are quaternary salts as well as to those with longer side-chains.

Relationships between $\triangle pK_a$, affinity and activity

(1) *Affinity.* In Figs. 3A, B, C, D and E, the values of $\triangle pK_a$ are plotted against log K for the antagonists and partial agonists and against the logarithm of the equipotent molar ratio relative to β-pyridylmethyltrimethylammonium for the agonists. The scale for the agonists has been inverted so that the more active compounds appear at the top of the figure.

Although it can be seen that substituents have large effects on affinity, these do not appear to be related to their effects on the pK_a of benzyldiethylamine. The complex pattern of results indicates that a number of other factors must be important, besides the effects of a substituent on electron distribution ; groups with similar electronic effects can be seen to have quite different effects of affinity.

The effects of substituents on affinity are, in fact, in many instances greater than the effects of the benzene ring itself. Barlow *et al.* (1967) found log K for methyltriethylammonium on the frog rectus (*Rana pipiens*) to be 2·45, compared with our value of 4·03 for benzyltriethylammonium. This indicates that the benzene ring contributes 2·2 kcal/mole to the free energy of adsorption. In contrast, much bigger differences in log K may be produced by altering the substituent on the benzene ring. The values of log K range from 2·14, for *o*-methoxybenzyldiethylamine, to 5·90 for *p*-hydroxybenzyldiethylamine, indicating that the free energy of adsorption of these compounds differs by over 5 kcal/mole.

In view of this, it seems highly unlikely that substituents exert their effects on affinity only by modifying electron distribution in the aromatic ring. The changes in charge distribution produced would not appear likely to be big enough to produce changes in affinity as big and as irregular as those observed. It seems much more likely that the effect of a group on affinity depends largely upon its direct interaction

with the receptor surface and that there are stringent steric requirements which affect these.

The importance of steric factors is shown by the irregular variation of affinity with the number of methylene groups in the unsubstituted compounds (Table 4). The lower affinity of phenethyltriethylammonium compared with the benzyl and phenylpropyl homologues is particularly striking. In part such differences in affinity may be associated with differences in flexibility, such as were suggested for pyridylmethyl and pyridylethyl compounds by Barlow & Hamilton (1962). However, although the benzyl compounds are undoubtedly much less flexible than the phenethyl compounds, it seems unlikely that differences in preferred conformation alone could account for the results shown in Table 4. The same compounds were tested by Scott (1967) as antagonists of acetylcholine on the isolated guinea-pig ileum in the presence of hexamethonium and the affinity increased in a regular manner, the values of log K being 4·83, 4·95, 5·18, 5·48 and 5·77 for the phenylalkyltriethylammonium compounds with one, two, three, four and five methylene groups respectively.

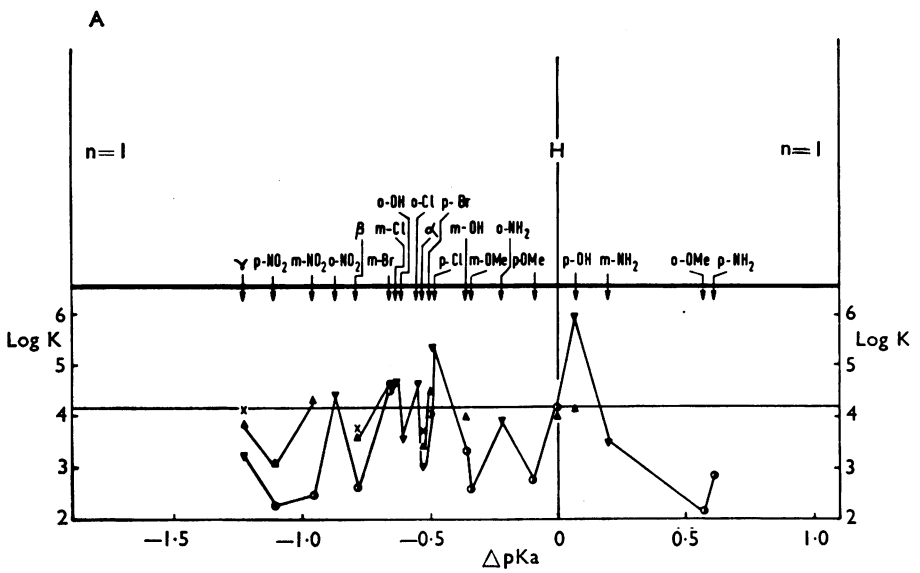


FIG. 3. Effect of substituent on biological activity. Ordinate: Lower section, log. affinity constant; upper section, log. equipotent molar ratio relative to β-pyridylmethyltrimethylammonium, with the scale inverted so that the more active compounds appear at the top; abscissa: effect of substituent on electron distribution, $\Delta pK_a = pK_a$ benzyldiethylamine $- pK_a$ substituted benzyldiethylamine or pyridylmethyldiethylamine (indicated by α, β and γ; all pK_a measurements were made in 40% ethanol). Note that the division between the upper and lower sections is quite arbitrary because the affinity of the agonists is not known, nor its relationship to equipotent molar ratio.

□ indicates $\overset{+}{N}Me_3$ and agonist; ○ indicates $\overset{+}{N}Me_3$ and partial agonist; △ indicates $\overset{+}{N}Me_3$ and antagonist; ▼ indicates $\overset{+}{N}Et_2H$ and antagonist; ◑ indicates $\overset{+}{N}Et_2H$ and partial agonist; ▲ indicates $\overset{+}{N}Et_3$ and antagonist; ● indicates $\overset{+}{N}Et_3$ and partial agonist; ■ indicates $\overset{+}{N}Et_3$ and agonist; × indicates $\overset{+}{N}nPr_2H$ and antagonist.

Results for the compounds with one methylene group are shown in sections A and B, for those with two methylene groups in sections C and D and for those with three methylene groups in section E.

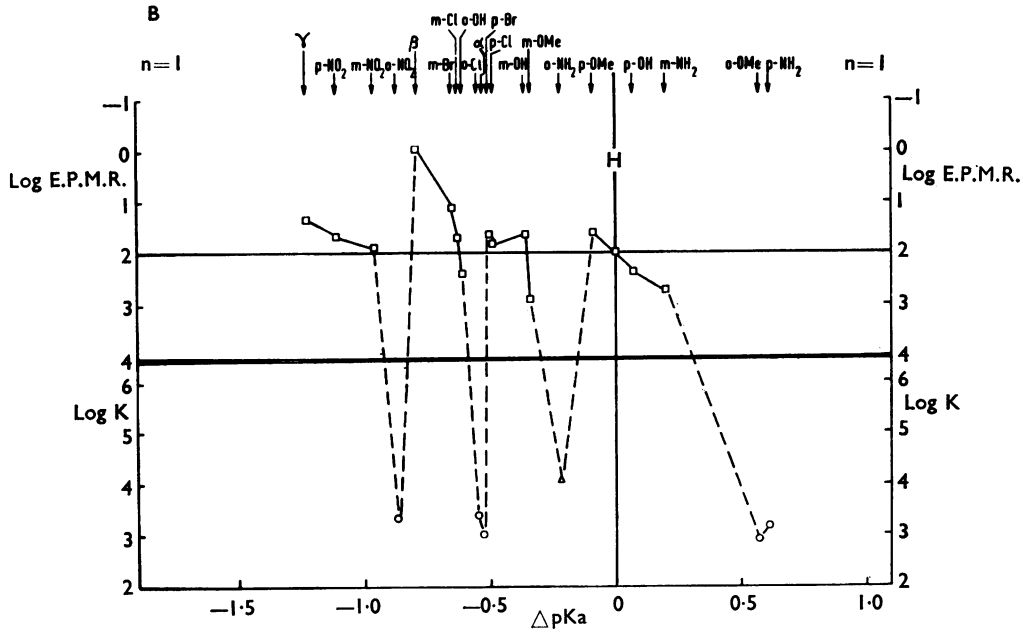


FIG. 3. B. See legend on page 569.

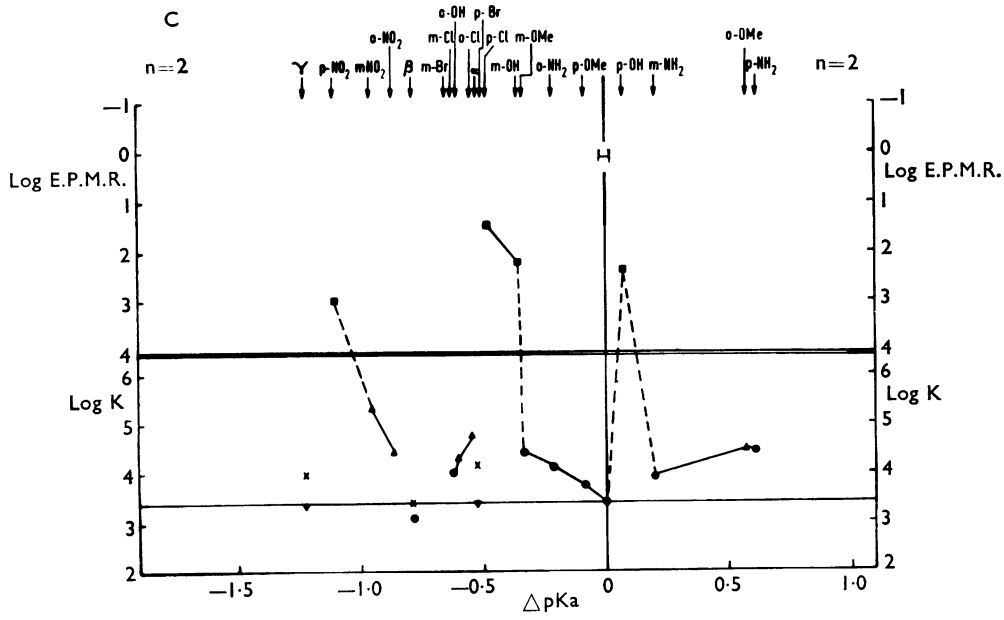


FIG. 3. C. See legend on page 569.

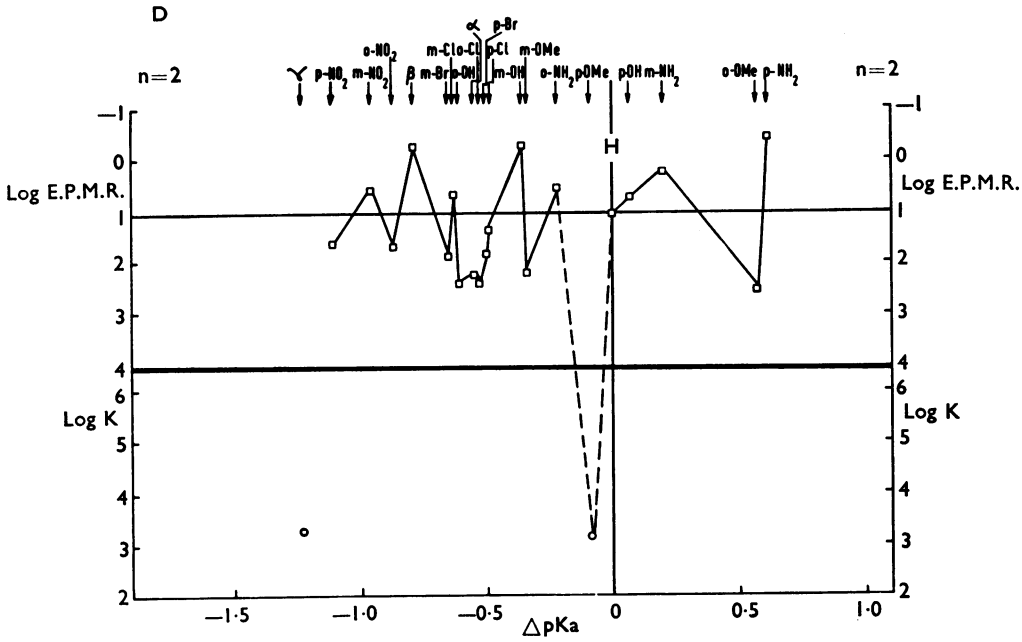


FIG. 3. D. See legend on page 569.

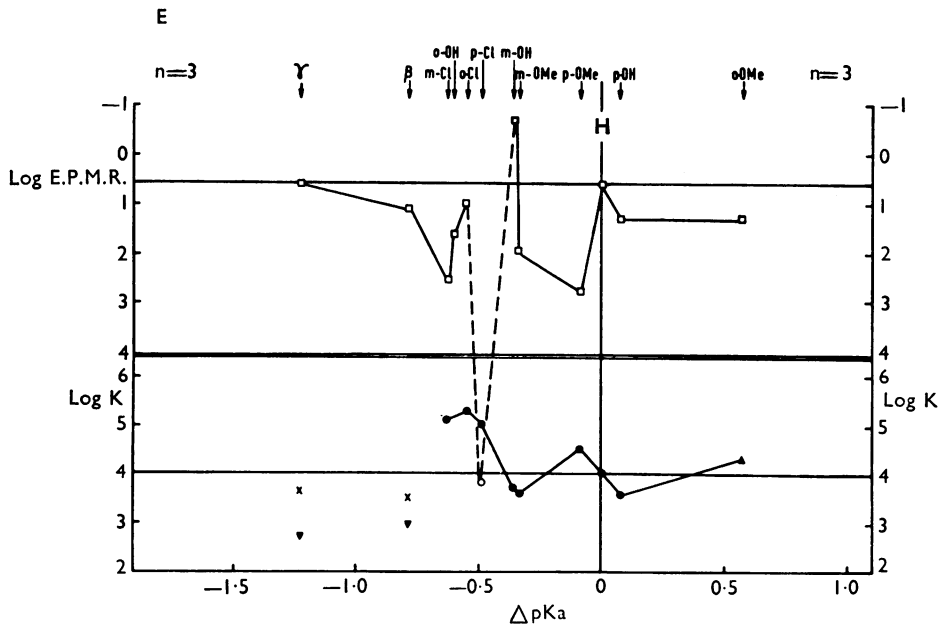


FIG. 3. E. See legend on page 569.

The critical importance of steric factors in relationships between chemical structure and affinity for these receptors is emphasized by the variable effects on affinity of increasing the size of the onium group. With the pyridylmethyl compounds, an increase in the size of the cationic head from diethylamine to triethylammonium and di-*n*-propylamine leads to an increase in affinity (Fig. 3A). In the nitro- and bromo- benzyl series, too, the triethylammonium compounds have greater affinity than the tertiary bases. On the other hand, benzyl, phenethyl and phenylpropyl diethylamines all have higher affinity than their triethylammonium analogues and *p*-hydroxybenzyltriethylammonium has only moderate affinity, in contrast to the very high affinity of the analogous tertiary base.

The irregular effects on affinity of quaternization suggest that alterations in the onium group disturb the binding of the rest of the molecule, just as has been observed with series of compounds tested at acetylcholine receptors in the guinea-pig ileum (Abramson *et al.*, 1969). Members of a series of chemically similar compounds, substituted benzyldiethylamines, for example, are probably not all bound at the receptor with the onium group in exactly the same position relative to the group in the receptor with which it interacts. The effects of a substituent in the benzene ring on affinity will be made up of at least two components, its own contribution to binding and its effect on the binding of the rest of the molecule, particularly that of the onium group.

In an attempt to see what factors determine the effects of a group on affinity, values of $\triangle$log K have been calculated by subtracting log K of the unsubstituted phenyl compounds from that of the substituted derivatives (Table 6). By making comparisons in this way between compounds with the same number of methylene groups it should be possible to see the effects of *m*- and *p*- substituents free from any effects which arise from differences in flexibility. With *o*-compounds, however,

TABLE 6. *Effect of substituent on affinity.* $\triangle$log K = log K of substituted compound − log K of unsubstituted compound; that is, positive values indicate increased affinity. A query indicates that the compound is a pure agonist whose affinity is not known.

		n=1			n=2		n=3
Substituent		$\overset{+}{N}Et_2H$	$\overset{+}{N}Et_3$		$\overset{+}{N}Et_3$		$\overset{+}{N}Et_3$
NO_2	*o*	+0·26	—		+1·02		—
	m	*−1·69*	+0·30		+1·91		—
	p	*−1·88*	−0·92		?		—
Cl	*o*	+0·50	—		+1·31		*+1·26*
	m	+0·54	—		*+0·61*		*+1·12*
	p	+1·22	—		?		*+0·99*
Br	*o*	—	—		—		—
	m	*+0·41*	*+0·64*		—		—
	p	*−0·10*	+0·46				
MeO	*o*	*−2·01*	—		+1·05		+0·29
	m	*−1·58*	—		*+1·03*		*−0·37*
	p	*−1·42*	—		*+0·37*		*+0·48*
HO	*o*	−0·61	—		+0·88		+0·40
	m	*−0·84*	−0·07		?		*−0·31*
	p	+1·75	+0·14		?		*−0·45*
NH_2	*o*	−0·22	—		*+0·69*		—
	m	−0·68	—		*+0·52*		—
	p	*−1·31*	—		*+1·03*		—
Pyridyl				$\overset{+}{N}nPr_2H$	$\overset{+}{N}Et_2H$	$\overset{+}{N}nPr_2H$	$\overset{+}{N}Et_2H$
	α	−1·13	−0·62	−0·85	−0·77	−0·05	—
	β	−1·51	−0·35	−0·87	−1·09	−0·79	−1·50
	γ	−0·88	−0·17	−0·45	−0·84	−0·23	−1·71

The italic figure values involve results obtained from partial agonists.

particularly those with only one methylene group, the substituent itself may have a marked effect on flexibility. Chloro groups appear always to increase affinity. Most other groups reduce affinity in the benzyldiethylamines but, with the exception of pyridyl, increase affinity in the compounds with two methylene groups.

It is difficult to discern any regular pattern, and there seems to be no particular reason why *p*-hydroxybenzyldiethylamine should have such high affinity. In addition to the effects of substituents on electron distribution, it might be expected that their size would be important, but there is no obvious correlation between size and $\triangle$log K ; the chloro group is intermediate in size compared with the others which were tested. The lipophilicity of a substituent might also be important. Even though our measurements of log K were made in conditions in which the antagonists had time to come into equilibrium with the receptors, and so should be independent of differences in rates of redistribution (or of diffusion), the ability of a substituent to promote solubility in lipid might be an indication of the extent to which it would promote hydrophobic bonding between drug and receptor. There is no obvious correlation, however, between $\triangle$log K and the parameter π, used by Fujita, Iwasa & Hansch (1964) to assess lipophilic activity.

It seems that the effects of a substituent on affinity probably depend on a number of factors. These may include its effect on electron distribution and its lipophilicity, but are probably determined primarily by the polarization of the group itself, its size, and its position relative to the onium group. In addition it may produce effects by altering the preferred conformations of molecules, particularly if it is in the *o*-position.

(2) *Activity*

In contrast to the uncertainty about relationships between affinity and structure, certain chemical features are clearly associated with activity. In Fig. 3B it can be seen that in the compounds with one methylene group six of the trimethylammonium derivatives are only partial agonists. Five of these are *o*-substituted, the sixth is the *p*-amino compound. In the compounds with two methylene groups (Fig. 3A), however, four of the triethylammonium compounds are agonists, those with *p*-nitro, *p*-chloro and *p*- and *m*-hydroxy groups. Of the trimethylammonium compounds (Fig. 3A), only the *p*-methoxy and γ-pyridyl compounds are partial agonists. It appears, therefore, that the presence of two methylene groups in the side-chain is associated with efficacy. Of the trimethylammonium compounds with three methylene groups which were studied (Fig. 3C), the *p*-chloro compound is only a partial agonist.

For *p*-chloro compounds it appears that there is a decline in efficacy when the number of methylene groups is increased from two to three, and this is true also for phenylalkyl and pyridylalkyl-diethylamines, in which series the compounds with two methylene groups are partial agonists, whereas those with three are antagonists. In the *m*-hydroxy compounds, too, there appears to be a decline in efficacy when the number of methylene groups is increased from two to three. This may only be small however, because *m*-hydroxyphenethyltriethylammonium is only a feeble agonist, and *m*-hydroxyphenylpropyltriethylammonium is quite a strong partial agonist. Of the other compounds with three methylene groups which have been studied, the *o*-hydroxy, *o*-methoxy and *o*-chloro compounds are more active relative to the compounds with *m*- and *p*- substituents than they are in the series with one

or two methylene groups. This rise in efficacy might mean that the *o*-substituents are better able to interact with groups in the receptor when they are further from the onium group. The compounds do not differ greatly in activity from the unsubstituted derivatives, however, and it seems more probable that *o*-substituents reduce efficacy in the compounds with shorter side chains by reducing the flexibility of the molecule.

The effect of chain length on the activity of the phenylalkyltrimethylammonium compounds (Table 4) is not a simple relationship but shows two maxima, one with the trimethylene compound and a second, smaller, one with the pentamethylene compound. Similar results were observed with the phenoxyalkyltrimethylammonium compounds studied by Hamilton (results quoted by Barlow, 1965), though the second maximum occurred with phenoxypentyltrimethylammonium, which would correspond to phenylhexyltrimethylammonium (which is a partial agonist). It is surprising that the ethers are less active than the alkanes, but the results could be explained if the alkanes have higher affinity and lower efficacy than the ethers. It would be interesting to compare the affinity of the phenoxyalkyl and phenylalkyl triethylammonium compounds.

The effects of the size of the onium group are similar to those observed with simple onium salts by Barlow *et al.* (1967). There is a transition from agonist to partial agonist and antagonist as the size is increased, for example, by the successive replacement of methyl groups in β-pyridylmethyltrimethylammonium by ethyl groups (Table 2), indicating a loss of efficacy. As increasing the size of the onium group usually increases affinity, it appears that with these compounds, as with the simple onium salts, changes which increase affinity are not usually associated with increases in activity.

The effects of substituents on activity can be seen in Table 7. The figures shown

TABLE 7. *Effect of substituent on activity of trimethylammonium compounds.* △ log equipotent molar ratio = log. equipotent molar ratio of unsubstituted compound − log equipotent molar ratio of substituted compound; positive values indicate increased activity.

Substituent		n=1		n=2		n=3	
NO_2	*o*	*		−0·66	(+1·02)	—	
	m	+0·09	(+0·30)	+0·43	(+1·91)	—	
	p	+0·30	(−0·92)	−0·61		—	
Cl	*o*	*		−1·25	(+1·31)	−0·43	(+1·26)
	m	+0·26		+0·35	(+0·61)	−1·99	(+1·12)
	p	+0·17		−0·35		*	
Br	*o*	—		—		—	
	m	+0·85		−0·85		—	
	p	+0·33		−0·80		—	
MeO	*o*	*		−1·52	(+1·05)	−0·74	(+0·29)
	m	−0·92		−1·17	(+1·03)	−1·38	(−0·37)
	p	+0·35		*		−2·22	(+0·48)
HO	*o*	−0·43		−1·37	(+0·88)	−1·05	(+0·40)
	m	+0·30	(−0·07)	+1·30		+1·28	(−0·31)
	p	−0·40	(+0·14)	+0·40		−0·72	(−0·45)
NH_2	*o*	*		+0·49	(+0·69)	—	
	m	−0·74		+0·79	(+0·52)	—	
	p	*		+1·47	(+1·03)	—	
Pyridyl							
	α	*	(−0·62)	−1·40		—	
	β	+1·97	(−0 35)	+1·28		−0·50	
	γ	+0·62	(−0·17)	*		−0·11	

Values in parentheses show the effects of the substituent on log K for the analogous triethylammonium compounds (Table 6).
An asterisk indicates that the compound is not an agonist.

are △log. equipotent molar ratio—the logarithm of the equipotent molar ratio of the unsubstituted phenylalkyltrimethylammonium compound *minus* the logarithm of the equipotent molar ratio for the substituted compound, and are therefore positive if activity is increased by the substituent. A comparison of Tables 6 and 7 does not reveal any obvious connexion between the effects of a substituent on affinity and its effects on activity. Some substituents with positive values in Table 6—for example, the methoxyphenethyl compounds—have negative values in Table 7. Others, on the other hand—for example, the pyridylmethyl compounds—have positive values in Table 7 and negative values in Table 6. This appears to support the idea that high affinity may be incompatible with high efficacy, as was suggested by Stephenson (1956) from results on the isolated guinea-pig ileum, and supported by results with simple onium salts on the frog rectus (Barlow *et al.*, 1967). It does not appear to be entirely correct, however, because there are compounds such as the aminophenethyl derivatives which have positive values in both Tables 6 and 7.

The three types of substituent which are particularly associated with activity, hydroxy, amino and pyridyl, differ markedly in their effects on electron distribution but are all capable of forming hydrogen bonds. It seems unlikely, however, that the high activity of compounds containing these groups is due to their being bound to the receptor surface by hydrogen bonds. For bonding with pyridyl, the group on the receptor would have to contain hydrogen, whereas the other groups contain hydrogen themselves. If the action of the drug at the receptor is brought about by its ability to cause a change in the structure of the membrane, a possible explanation of the results might be that this change involves water molecules associated with a particular group within the receptor. Groups which interact with water, such as amino, hydroxyl, and pyridyl, may confer efficacy, as will cationic groups of a suitable size. Activity would then be highest in those molecules in which adsorption at the receptor allows the groups the best opportunity to interact with these water molecules. It may be possible to increase adsorption by introducing substituents which interact with other parts of the receptor, but this will only lead to increased activity if the binding of the molecule to the receptor is not disturbed, so that there is no loss of efficacy. This is unlikely, but not impossible, and would explain what we have found, that substituents which usually increase affinity decrease activity but do not always do so.

Compounds

The melting points and analyses of the compounds used in this work are shown in Table 8. Section A refers to the pyridyl compounds, section B to the substituted phenyl compounds and section C to the unsubstituted phenylalkyl compounds.

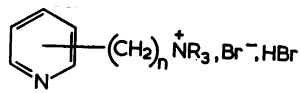
The pyridyl derivatives were prepared by the methods of Barlow & Hamilton (1962), which have been extended to include pyridylpropanols, now commercially available, as well as different secondary and tertiary bases from those used originally. Improved yields of the pyridylalkylchloride hydrochlorides were obtained by adding the alcohol to thionyl chloride at room temperature without any solvent, instead of as previously described. The mixture was heated until no more sulphur dioxide was evolved, and the unreacted thionyl chloride was distilled off under reduced pressure. This left the crude pyridylalkylchloride hydrochloride, which was either recrystallized or converted to the free pyridylalkylchloride, which was distilled if this was possible, and immediately added to the appropriate secondary or tertiary base.

TABLE 8. *Melting-points and analyses*

Melting-points were measured on a Kofler hot-stage microscope except those given to 0·1° C, which were recorded with a Mettler FP 1 instrument, coupled to a pen recorder, with a rate of heating of 0·2° C/min. All analyses are for ionized halogen, measured gravimetrically, with samples of at least 100 mg (Barlow & Zoller, 1965). The compounds were crystallized from combinations of methanol, ethanol, ethylmethylketone and ethylacetate; with the more soluble ones it was necessary to add ethylacetate to a concentrated solution in ethylmethyl ketone, but the less soluble ones crystallized satisfactorily from ethanol and with one or two particularly insoluble compounds it was necessary to use methanol. A dash indicates that the compound was not prepared. Glässer & Pasini (1960) do not record the m.p. of leptodactyline iodide, but the picrate had m.p. 200°–202°. From the iodide, m.p. 164·1°–165·2°, we obtained a picrate m.p. 198·4°–198·9°.

A. *Pyridyl compounds*

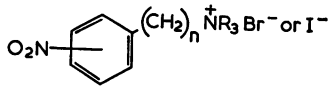
The compound marked with an asterisk appeared to contain water of crystallization, and lost weight, but also began to decompose when dried at 60°/0·1 mm with phosphorus pentoxide; theory for $C_{11}H_{20}N_2Br_2$, $\frac{1}{2}H_2O$; Br^-, 45·8%.



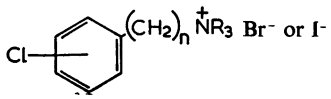
		α	β	γ	Theory %
n=1					
$\overset{+}{N}R_3=\overset{+}{N}Et_2H$	m.p.	177·5°–177·9°	201°–2°	dec. 213°–6°	
	Found Br^-	49·2	49·2	49·0	49·0
$\overset{+}{N}Et_3$	m.p.	160·8°–161·4°	191°–192·5°	dec. 241°	
	Found Br^-	44·9	44·9	45·1	45·1
$\overset{+}{N}nPr_2H$	m.p.	195°–6°	219°–220°	dec. 225°–7°	
	Found Br^-	45·0	45·2	45·3	45·1
$\overset{+}{N}nBu_2H$	m.p.	—	126°–7°	—	
	Found Br^-		42·0		41·8
n=2					
$\overset{+}{N}R_3=\overset{+}{N}Et_2H$	m.p.	190°–190·5°	158·8°–159·2°	dec. above 245°	
	Found Br^-	47·0	47·2	47·0	47·0
$\overset{+}{N}nPr_2H$	m.p.	183°–183·5°	172·6°–172·8°	dec. above 245°	
	Found Br^-	43·5	43·4	43·4	43·4
n=3					
$\overset{+}{N}R_3=\overset{+}{N}Me_2H$	m.p.	—	166·6°–167·1°	sinters 191·0°–191·4° melts 194·8°–195·4°	
	Found Br^-		48·9	49·3	49·0
$\overset{+}{N}Me_3$	m.p.	—	201·8°–202·6°	202·8°–203·4°	
	Found Br^-		46·0*	46·7	47·0
$\overset{+}{N}Et_2H$	m.p.	—	180·0°–180·6°	199·1°–199·7°	
	Found Br^-		45·0	45·2	45·1
$\overset{+}{N}nPr_2H$	m.p.	—	204·0°–204·6°	205·0°–205·5°	
	Found Br^-		41·7	42·1	41·8

Table 8 (continued)

B.

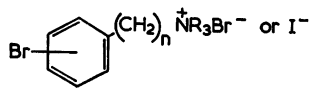


		o	*m*	*p*	Theory %
n=1					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	204°–5°	206°–206·5°	203°–4°	
	Found I^-	39·1	39·5	39·4	39·4
$\overset{+}{N}Et_2H$	m.p.	151°–152·5°	184°–190°	121·3°–121·9°	
	Found Br^-	27·6	27·9	27·5	27·6
$\overset{+}{N}Et_3$	m.p.	—	197°–8°	213°–4°	
	Found I^-		34·8		34·9
	Found Br^-			25·3	25·2
n=2					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	242°–5°	245°–6°	212°–4°	
	Found I^-	38·0	38·1	37·8	37·8
$\overset{+}{N}Et_3$	m.p.	167°–172°	211°–2°	168°–170°	
	Found I^-	33·6		33·9	33·6
	Found Br^-		24·0		24·2

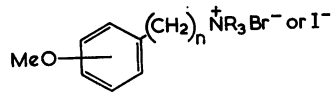


		o	*m*	*p*	Theory %
n=1					
$\overset{+}{N}R_3=\overset{+}{N}Me_2H$	m.p.	194·8°–195·2°	194·7°–195·2°	194·1°–194·4°	
	Found Br^-	31·8	32·1	32·2	31·9
$\overset{+}{N}Me_3$	m.p.	161·5°–162°	199°–200°	260°–261°	
	Found I^-	41·0	41·0	40·5	40·7
$\overset{+}{N}Et_2H$	m.p.	158·9°–159·4°	201·7°–202·6°	126·2°–127·3°	
	Found Br^-	28·8	28·8	28·6	28·8
$\overset{+}{N}Et_3$	m.p.	—	159°–160°	193·4°–194·4°	
	Found I^-		35·7	35·7	35·8
n=2					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	258°–261°	209°–210°	250°–253°	
	Found I^-	39·1	39·0	39·3	39·0
$\overset{+}{N}Et_3$	m.p.	185°–6°	108°–108·5°	250°–251°	
	Found I^-	34·8	34·5	34·8	34·5
n=3					
$\overset{+}{N}R_3=\overset{+}{N}Me_2H$	m.p.	144·4°–145·2°	127·6°–128·7°	130·8°–133·8°	
	Found Br^-	28·9	28·7	28·8	28·7
$\overset{+}{N}Me_3$	m.p.	137·0°–137·7°	102·1°–102·8°	154·4°–154·9°	
	Found Br^-	27·2	27·7	27·6	27·4
$\overset{+}{N}Et_2H$	m.p.	122·0°–122·6°	165·4°–166·3°	102·9°–103·9°	
	Found Br^-	26·3	26·1	26·2	26·1
$\overset{+}{N}Et_3$	m.p.	175·6°–175·9°	134·2°–134·9°	146·3°–147·1°	
	Found Br^-	24·2	23·8	23·9	23·9

Table 8 (continued)

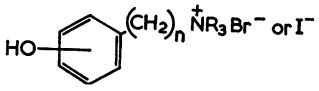


n=1		*o*	*m*	*p*	Theory %
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	—	208°–211°	233°–237°	
	Found I^-		35·4	35·7	35·7
$\overset{+}{N}Et_2H$	m.p.	—	193·8°–194·0°	151·0°–151·6°	
	Found Br^-		24·8	25·0	24·8
$\overset{+}{N}Et_3$	m.p.	—	189°–190°	186°–188°	
	Found I^-		31·8	32·1	31·9
n=2					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	181·2°–181·3°	136·5°–137·4°	195·1°–195·2°	
	Found I^-	34·4	34·2	34·2	34·5

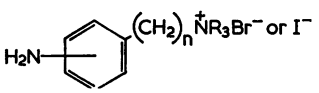


n=1		*o*	*m*	*p*	Theory %
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	162·2°–162·9°[c]	143·5°–144·3°	157·1°–158·1°	
	Found I^-	41·5	41·2	41·6	41·4
$\overset{+}{N}Et_2H$	m.p.	120·2°–121·0°	145·3°–146·0°	104·8°–105·6°	
	Found Br^-	29·5	29·4	29·2	29·2
n=2					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	211·3°–211·7°	182·3°–182·6°	211·4°–211·7°	
	Found I^-	39·5	39·6	39·9	39·6
$\overset{+}{N}Et_3$	m.p.	159·6°–160·3°	103·7°–104·2°	164·9°–165·3°	
	Found I^-	35·1	34·8	34·8	35·0
n=3					
$\overset{+}{N}R_3=\overset{+}{N}Me_3$	m.p.	138·0°–138·3°	87·4°–87·8°	125·1°–125·4°	
	Found I^-	37·7	37·8	37·7	37·9
$\overset{+}{N}Et_3$	m.p.	119·8°–120·5°	95·0°–96·0°	101·3°–101·7°	
	Found I^-	32·8	33·6	33·7	33·7

Table 8 (continued)



n=1		*o*	*m*	*p*	Theory %
$R_3\overset{+}{N}=\overset{+}{N}Me_3$	m.p.	171·4°–171·5°	171·1°–171·8°	184°–186°	
	Found I^-	43·4	43·6	43·6	43·3
$\overset{+}{N}Et_2H$	m.p.	255°–257°	131·1°–131·9°	151·7°–152·3°	
	Found Br^-	31·0	30·9	30·5	30·8
$\overset{+}{N}Et_3$	m.p.	—	139°–140°	175·4°–175·5°	
	Found I^-		37·8		37·9
	Found Br^-			27·9	27·8
n=2					
$R_3\overset{+}{N}=\overset{+}{N}Me_3$	m.p.	226·2°–226·5°	164·1°–165·2°	227·4°–227·7°	
	Found I^-	41·2	41·6	41·6	41·4
$\overset{+}{N}Et_3$	m.p.	181·6°–182·2°	167·7°–168·3°	214·7°–215·0°	
	Found I^-	36·5	36·4	36·3	36·4
n=3					
$R_3\overset{+}{N}=\overset{+}{N}Me_3$	m.p.	174·0°–175·2°	156·0°–156·9°	208·3°–208·7°	
	Found I^-	39·2	39·6	39·6	39·6
$\overset{+}{N}Et_3$	m.p.	140·9°–141·4°	141·7°–142·1°	166·2°–166·4°	
	Found I^-	34·8	35·3	35·0	35·0



n=1		*o*	*m*	*p*	Theory %
$R_3\overset{+}{N}=\overset{+}{N}Me_3$	m.p.	170·2°–170·4°	157·0°–157·5°	140·0°–140·2°	
	Found I^-	43·7	43·2	43·4	43·5
$\overset{+}{N}Et_2H$	m.p.	sublimes 172°–180°	215·7°–216·6°	186°–190°	
	Found Br^-	47·3	47·1	47·3	47·0
n=2					
$R_3\overset{+}{N}=\overset{+}{N}Me_3$	m.p.	218°–220°	204°–206°	220°–221°	
	Found I^-	41·8	41·4	41·5	41·5
$\overset{+}{N}Et_3$	m.p.	204·6°–204·8°	152°–153°	224·9°–225·2°	
	Found I^-	36·7	36·3	36·1	36·5

(*Continued on page* 580)

α-Pyridylmethylchloride had b.p. 35°–36°/0·15 mm, N_D^{25}, 1·5356; β-pyridylmethylchloride had b.p. 40°/0·05 mm, N_D^{25}, 1·5412; γ-pyridylmethylchloride had b.p. 45°/0·12 mm, N_D^{25}, 1·5428 but decomposed partly during distillation.

α-Pyridylethylchloride had b.p. 62°–63°/0·5 mm, N_D^{25}, 1·5261; β-pyridylethylchloride had b.p. 109°/12 mm, N_D^{25}, 1·5337; we were unable to make the γ-isomer.

β-Pyridylpropylchloride had b.p. 69°–75°/0·12 mm, N_D^{25}, 1·5249; γ-pyridylpropylchloride had b.p. 79°–83°/0·15 mm, N_D^{25}, 1·5253; the α-isomer decomposed when distilled.

Because γ-pyridylethylchloride could not be obtained, either as a free base or as its hydrochloride, it was not possible to prepare γ-pyridylethyltrimethylammonium bromide hydrobromide with trimethylamine in the usual way. An attempt was made to make γ-pyridylethyltrimethylammonium iodide by treating γ-pyridylethyldimethylamine, obtained by the addition of dimethylamine to 4-vinylpyridine, with methyl iodide. The same conditions were chosen as have been used for the preparation of nicotine monomethiodide (Pictet & Genequand, 1897; Barlow & Dobson, 1955). The tertiary base (15 g, 0·1 mole) was dissolved in methanol (150 ml.), methyl iodide (10 g, 0·08 mole) was added and the mixture left overnight and then heated under reflux for half an hour. A mixture of products was obtained, which included the *iso*-methiodide, which has the pyridine ring methylated, as well as the desired monomethiodide and the dimethiodide. The mono- and iso-methiodides were separated from the dimethiodide by extracting the mixture with ethylmethyl ketone, in which the dimethiodide was not very soluble. The solution in ethylmethyl

---

Table 8 (*continued from page 579*)

C.

$Ph(CH_2)_n\overset{+}{N}R_3\ Br^-$

	m.p.	Found Br^-	Theory %
$\overset{+}{N}R_3 = Me_3\overset{+}{N}$			
n=2	242°–3°	32·5	32·8
3	151°–2°	30·9	30·9
4	184°–5°	29·3	29·4
5	168°–9°	27·9	27·9
6	182·6°–183·0°	26·5	26·7
7	173·3°–173·5°	25·4	25·5
$\overset{+}{N}R_3 = Et_3\overset{+}{N}$			
n=1	196·1°–196·6°	29·4	29·4
2	134·8°–135·3°	28·1	28·0
3	99·4°–101·1°	26·7	26·7
4	85·8°–86·6°	25·5	25·5
5	118°–9°	24·2	24·4
6	69·7°–70·2°	23·1	23·4
7	66·6°–68·5°	22·2	22·5
$\overset{+}{N}R_3 = Et_2\overset{+}{N}H$			
n=1	160·8°–161·4°	32·8	32·8
2	106·5°–107·2°	31·1	31·1
3	142·0°–142·7°	29·4	29·4
$\overset{+}{N}R_3 = n Pr_2\overset{+}{N}H$			
n=1	198·3°–198·4°	29·3	29·4
2	122·9°–123·8°	28·3	28·0

ketone was concentrated under reduced pressure, dissolved in a mixture of butanol, ethanol and water (5:5:2 by volume) and passed down a column of Sephadex LH 20. The main bulk of material consisted of a fraction from which was obtained a solid which decomposed at 151°–152°, found I^-, 47·6; $C_{10}H_{17}N_2I$ requires I^-, 43·4%. The ultraviolet absorption spectrum in water had λmax 256 mμ, with a shoulder at 261–263 mμ (ε, 2·35 and $1·7 \times 10^3$ respectively). γ-Pyridylethyldimethylamine monohydrogen iodide, m.p. 178–9°, prepared by adding one mole of hydriodic acid to the tertiary base, had λmax 255 mμ, with a shoulder at 262 mμ (ε, 2·2 and $1·5 \times 10^3$ respectively). It seems likely that we have obtained the monomethiodide, contaminated with traces of trimethylamine hydrogen iodide, formed by decomposition. It is clear from the ease with which solutions containing the material turn brown and smelt of 4-vinyl pyridine that the compound is not particularly stable, and it was found that after storage for 6 months the solid decomposed at 149°–150° instead of at 151°–152°, even though it had been kept in a dark bottle in a dessicator.

The other pyridyl compounds used in this work not included in Table 8A have been described by Barlow & Hamilton (1962). It was necessary to repeat the synthesis of some of them and it was found that their melting-points were slightly lower when recorded with the Mettler FP 1 instrument than with the Kofler hot-stage. β-Pyridylethyltrimethylammonium bromide hydrobromide had m.p. 217·8°–218·2°, compared with 226°–7°, and the difference was not due to impurities (found Br^-, 49·0; theory 49·0; compare 48·5%, Barlow & Hamilton, 1962). The difference in melting-point was usually small and probably arises because the rate of heating with the Mettler instrument is slower than with the Kofler hot-stage microscope. With α-pyridylethyltrimethylammonium bromide hydrobromide, however, it was considerable, 148·5°–149·0°, as compared with 180°–181°. The analysis of the fresh batch was satisfactory, found Br^-, 49·2, theory 49·0% and it seems that the compound sinters at the lower temperature but does not melt completely until 180°.

Substituted benzylamines

The appropriate aldehyde was reduced to the alcohol, converted to the bromide and thence to the substituted dimethylamine or diethylamine. This was quaternized with methyl or ethyl iodide. With *o*-substituted compounds, however, it was not found possible to obtain the substituted triethylammonium salts in this way, though the trimethylammonium compounds were prepared successfully. The amino-substituted benzyldialkylamines were made from the corresponding nitro-compounds by reduction in ethanol with hydrogen and platinum-charcoal. The hydroxy compounds were made by demethylating the corresponding methoxy compounds by heating them with hydriodic or hydrobromic acid under reflux.

Substituted phenethylamines

The chloro- and methoxy-compounds were made from the appropriate benzyl bromide, which was converted to the cyanide and reduced to the amine with lithium aluminium hydride. This was methylated with formaldehyde and formic acid and the purified tertiary base was then quaternized with methyl iodide. The triethylammonium compounds were prepared from the primary amine by heating under reflux with ethyl iodide and potassium carbonate in a mixture of acetone and water,

without the isolation of the tertiary phenethyldiethylamine. The *o*- and *p*-hydroxyphenethyltrimethylammonium compounds were made by demethylating the corresponding methoxy tertiary bases, by heating them with hydriodic or hydrobromic acid under reflux. The hydroxy tertiary bases were then purified and methylated. The *m*-hydroxy compound (leptodactyline ; Erspamer, 1959) could not be obtained in this way but was prepared by demethylating *m*-methoxyphenethyltrimethylammonium iodide by heating it under reflux with hydriodic acid, as described by Glässer & Pasini (1960). The hydroxyphenethyltriethylammonium compounds were likewise made by demethylation of the corresponding methoxyphenethyltriethylammonium iodides.

The *o*- and *p*-nitro compounds were obtained by nitrating phenethyl bromide (Foreman & McElvain, 1940). The *o*- and *p*-nitrophenethyl bromides were then treated with dimethylamine or diethylamine and the tertiary base was quaternized with methyl or ethyl iodide. The tertiary bases were also reduced in ethanol with hydrogen and Raney nickel to the corresponding amino-phenethyl dimethyl- and diethyl- amines, which were then quaternized with methyl or ethyl iodide. The *m*-nitro compounds were obtained from *m*-nitrophenylpropionic acid, converted by way of the acid chloride to the amide, which was then treated with sodium hypobromite (compare Gulland, Hawarth & Virden, 1929, who used hypochlorite). The *m*-nitrophenethylamine obtained was then methylated with formaldehyde and formic acid, and the tertiary base was purified and quaternized with methyliodide. The corresponding triethylammonium compound was obtained by heating the primary amine under reflux with ethyliodide and potassium carbonate in a mixture of acetone and water, without the isolation of the tertiary phenethyldiethylamine. The iodide ion was exchanged for bromide with an ion exchange column. The *m*-amino compound was prepared from *m*-nitrophenylacetic acid, which was obtained from *m*-nitrobenzyl cyanide by the method used for the *p*-isomer by Robertson (1922), converted by way of the acid chloride to the NN-dimethyl and NN-diethyl amides. These were reduced in ethanol with Raney nickel and hydrogen to the aminophenyl derivatives and then with lithium aluminium hydride to the tertiary bases, which were quaternized with methyl or ethyl iodide.

The bromo compounds were obtained by converting the bromobenzyl bromide by way of the cyanide, acid, ester and alcohol to the bromophenethyl bromide. This was treated with dimethylamine or diethylamine and the tertiary base quaternized with methyl iodide. The attempts to quaternize bromophenethyldiethylamines with ethyl iodide, however, were unsuccessful.

Substituted phenylpropylamines

The chloro- and methoxy- compounds were all prepared from the substituted cinnamic acids, obtained from the benzaldehyde and malonic acid. The acids were esterified and reduced in ethanol with Raney nickel and hydrogen to the esters of the corresponding phenylpropionic acid, then with lithium aluminium hydride to the phenylpropanol ; this was converted to the bromide, which was treated with dimethylamine or diethylamine and the tertiary base quaternized with methyl or ethyl iodide. The hydroxy compounds were made by demethylating the corresponding methoxy quaternary salts by heating them with hydriodic acid under reflux.

Phenylalkyl trimethyl and triethylammonium salts

The phenylalkyl bromides were prepared by chain-lengthening reactions from benzylbromide and phenethylbromide, and were heated with trimethylamine or triethylamine in ethanol under reflux. Some of the trimethylammonium compounds have been prepared by Thomas & Marlow (1963), who recorded m.p. 238°–239° for the phenethyl compound, 152·0°–152·5° for the phenylpropyl compound, 181·5°–182·5° for the phenylbutyl compound and 163·5°–165·5° for the phenylpentyl compound.

We wish particularly to thank Mr. R. P. Stephenson, who designed the automated frog rectus apparatus, Miss Margaret Dalling and Miss Margaret Millar for competent technical help, and the Tobacco Research Council, whose very generous financial support made the work possible. The work was begun by Dr. N. C. Scott, who made measurements with many of the pyridyl compounds.

REFERENCES

ABRAMSON, F. B., BARLOW, R. B., MUSTAFA, M. G. & STEPHENSON, R. P. (1969). Relationships between chemical structure and affinity for acetylcholine receptors. *Br. J. Pharmac.*, **37**, 207–233.

ALBERT, A. (1968). *Selective Toxicity*, 4th ed., Appendix 3, pp. 460–463. London: Methuen.

ALBERT, A. & SERJEANT, E. P. (1962). *Ionisation Constants of Acids and Bases*, pp. 16–42. London: Methuen.

BARLOW, R. B. (1965). Chemical structure and biological activity of nicotine and related compounds. In *Tobacco Alkaloids and Related Compounds*, ed. von Euler, U. S., pp. 277–301. Oxford: Pergamon Press.

BARLOW, R. B. & DOBSON, N. A. (1955). Nicotine monomethiodide. *J. Pharm. Pharmac.*, **7**, 27–34

BARLOW, R. B. & HAMILTON, J. T. (1962). Effects of some isomers and analogues of nicotine on junctional transmission. *Br. J. Pharmac. Chemother.*, **18**, 510–542.

BARLOW, R. B. & MCLEOD, L. J. (1969). Some studies on cytisine and its methylated derivatives. *Br. J. Pharmac.*, **35**, 161–174.

BARLOW, R. B., SCOTT, N. C. & STEPHENSON, R. P. (1967). The affinity and efficacy of onium salts on the frog rectus abdominis. *Br. J. Pharmac. Chemother.*, **31**, 188–196.

BARLOW, R. B. & ZOLLER, A. (1965). Some effects of long chain polymethylene bis-onium salts on junctional transmission in the peripheral nervous system. *Br. J. Pharmac. Chemother.*, **23**, 131–150.

CLARK, J. & PERRIN, D. D. (1964). Prediction of the strengths of organic bases. *Q. Rev. chem. Soc.*, **18**, 295–320.

COLEMAN, M. E., HUME, A. H. & HOLLAND, W. C. (1965). Nicotine-like stimulant actions of several phenylcholine ethers. *J. Pharmac.*, **148**, 66–70.

ERSPAMER, V. (1959). Isolation of leptodactyline (m-hydroxy-phenylethyltrimethylammonium) from extracts of skin of Leptodactylus. *Arch. Biochem.*, **82**, 431–438.

ERSPAMER, V. & GLÄSSER, A. (1960). The pharmacological actions of *m*-hydroxyphenethyltrimethylammonium (Leptodactyline). *Br. J. Pharmac. Chemother.*, **15**, 14–22.

FOREMAN, E. L. & MCELVAIN, S. M. (1940). The reaction of organic halides with piperidine. *J. Am. chem. Soc.*, **62**, 1435–1438.

FUJITA, T., IWASA, J. & HANSCH, C. (1964). A new substituent constant, π, derived from partition coefficients. *J. Am. chem. Soc.*, **86**, 5175–5180.

GLÄSSER, A. & PASINI, C. (1960). Azioni farmacologiche di alcuni composti chimicamente correlati con la leptodactylina. *Il Farcamo*, **15**, 493–501.

GULLAND, J. M., HAWARTH, R. D. & VIRDEN, C. J. (1929). Synthetic experiments on the aporphine alkaloids. *J. chem. Soc.*, 1666–1676.

HAGLID, F. (1965). *Synthetic Analogues of Nicotine in Tobacco Alkaloids and Related Compounds*, ed. von Euler, U. S., pp. 213–319. Oxford: Pergamon Press.

HAGLID, F. (1967). Studies on pyridine alkaloids and their analogues. *Acta Pharm. Suec.*, **4**, 117–138.

HAMMETT, L. P. (1937). The effects of structure upon the reactions of organic compounds. Benzene derivatives. *J. Am. chem. Soc.*, **59**, 96–103.

HEY, P. (1952). On relationships between structure and nitotine-like stimulant activity in choline esters and ethers. *Br. J. Pharmac. Chemother.*, **7**, 117–129.

KIER, L. B. (1968). A molecular orbital calculation of the preferred conformation of nicotine. *Mol. Pharmac.*, **4**, 70–76.

ORMEROD, W. E. (1956). The pharmacology of benzoylcholine derivatives and the nature of carbonyl receptors. *Br. J. Pharmac. Chemother.*, **11**, 267–272.

PERRIN, D. D. (1965). *Dissociation Constants of Organic Bases in Aqueous Solution.* London: Butterworths.

PICTET, A. & GENEQUAND, P. (1897). Ueber die Jodmethylate des Nicotins. *Ber. dtsch. chem. Ges.*, **30**, 2117–2125.

ROBERTSON, G. R. (1922). *p*-Nitrophenylacetic acid. *Org. Synth.*, **2**, 59.

SCOTT, N. C. (1967). Ph.D. thesis, Univ. Edinburgh. A pharmacological study of compounds related to acetylcholine in order to investigate the effects of changes in chemical structure on affinity for receptors and the efficacy of the drug-receptor complex.

SEKUL, A. A. & HOLLAND, W. C. (1961a). Pharmacology of senecioylcholine. *J. Pharmac.*, **132**, 171–175.

SEKUL, A. A. & HOLLAND, W. C. (1961b). Comparative pressor effect of certain unsaturated esters of choline. *J. Pharmac.*, **133**, 313–318.

STEPHENSON, R. P. (1956). A modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11**, 379–393.

THOMAS, J. & MARLOW, W. (1963). Quaternary ammonium compounds. III. Antiacetylcholine activity and charge distribution in aromatic quaternary ammonium compounds. *J. med. Chem.*, **6**, 107–111.

WELLS, P. R. (1963). Linear free energy relationships. *Chem. Rev.*, **63**, 171–219.

WELLS, P. R. (1968). *Linear Free Energy Relationships.* London: Academic Press Inc. (London) Ltd.

WONG, K. C. & LONG, J. P. (1962). Nicotinic and muscarinic activity of phenacyl and phenalkyl trimethylamines. *J. Pharmac.*, **137**, 70–75.

(*Received April* 30, 1969)