17, 41, 72, and 145 hr. After heating, the samples were shown to have unchanged v.p.c. retention times and infrared and mass spectra consistent with deuterated allylacetophenone. N.m.r. analysis showed the D distributions listed in Table I.

Table I. Deuterium Distributions in Allylacetophenone

Time, hr.	CH_2(a)	CH_2(b)	CH(c)	CH_2(d)
17	1.73	0.00	0.00	0.20
41	1.37	0.00	0.00	0.56
72	1.23	0.00	0.00	0.74
145	0.97	0.00	0.00	0.90

(α-Methylallyl)acetophenone (**1**, CH_3) was prepared.[3b] The H n.m.r. absorptions of this compound are also nicely separated, except for one overlap that was not troublesome[5]: C_6H_5, 2 H(*o*) multiplet, τ 2.1, 3 H(*m,p*) multiplet, 2.6; CH_2(a) + CH(b) multiplet, 7.1; CH(c) multiplet, 4.1; CH_2(d) triplet, 5.0; CH_3(e) doublet, 8.9, J = 7 c.p.s. The hydrogens on the α-carbon of **1** (CH_3) were exchanged for D as before.[4] The n.m.r. spectrum of **2** (CH_3) was identical with that of **1** (CH_3) except for the much-reduced absorption centered at τ 7.1.[6] Intramolecular hydrogen exchange in this molecule according to the scheme **2** (CH_3) $\rightleftarrows$ **8** $\rightleftarrows$ **9** (CH_3) may be seen to correspond to the allylic phenol rearrangements demonstrated with deuterium labeling[2] and C^{14}-labeling,[7,8] and to be strictly analogous to those in which chemically different molecules are produced.[9,10] The expected equilibrium distribution of the two D's would be CH_2(a) 0.57, CH_2(d) 0.57, and CH_3(e) 0.86.

The deuterated compound **2** (CH_3) was heated at 200 ± 5° for 12, 48, and 121 hr. After heating, the samples were shown to have unchanged v.p.c. retention times and infrared and mass spectra consistent with the structure of deuterated (α-methylallyl)acetophenone. N.m.r. analysis showed the D distributions given in Table II.

Table II. Deuterium Distributions in (α-Methylallyl)acetophenone

Time, hr.	CH_2(a)	CH(c)	CH_2(d)	CH_3(e)
12	1.27	0.00	0.60	0.12
48	0.83	0.00	0.63	0.52
121	0.58	0.00	0.63	0.66

A direct comparison of our results with those of Schmid[2] cannot be made, since the experimental conditions were quite different. However, it is clear that D is transferred from the α-carbon methylene [CH_2(a)] exclusively to the terminal methylene groups [CH_2(d)] and to the methyl group [CH_3(e)], and that the rate of incorporation of D into CH_2(d) is much faster than into CH_3(e), as Schmid found.

Interestingly, there is a greater difference in the rates of D-methylene and D-methyl incorporation in our aliphatic system than in the phenol system: *e.g.*, in our 48-hr. experiment, with 59% migration of D from the α-carbon, $3n/2m$ = 1.82, while in the phenol system, with only 49% migration of D, $3n/2m$ = 1.78.[2] This is nicely explicable in terms of nonbonded interactions in the transition states for D-methyl incorporation into the two systems. Similar explanations can be offered for the slower rate of D transfer in allylacetophenone than in (α-methylallyl)acetophenone. These topics will be discussed in detail in the complete paper to be published later.

These preliminary results show clearly that reversible intramolecular hydrogen transfers are thermally induced between aliphatic allylic enol and cyclopropyl carbonyl systems. Among the topics of interest to us in our continuing study of these systems are the relationship of keto–enol equilibria and of steric and conformational effects to the rearrangements.

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(5) Since no D was found to migrate to the CH_2(b) in allylacetophenone, we felt justified in assuming no migration to the CH(b) in (α-methylallyl)acetophenone; one H was subtracted from the integrated signal from the CH_2(a) + CH(b) multiplet to give the H integral for CH_2(a).

(6) The H for D exchange was not complete. The n.m.r. spectrum of 2 (CH_3) showed 1.67 D and 0.33 H on the α-carbon[5] (mass spectrometry indicated 1.71 D). For simplicity, the numbers of D and H in various positions in the molecule after rearrangement, calculated from n.m.r. H integrals, were corrected to the values expected from 2.00 D originally on the α-carbon.

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A Correlation between the Biological Activity of Alkyltrimethylammonium Ions and Their Mode of Interaction with Acetylcholinesterase

Sir:

The transition from stimulant activity to antagonism attending the molecular modification of drugs is a phenomenon of considerable importance in medicinal chemistry. The physicochemical and biochemical parameters intervening in such transitions must be elucidated in order that structure–activity relationships among drugs may be interpreted. As a step in this direction we wish to report on the observation of a chain length dependent transition in the mode of binding of alkyltrimethylammonium ions on acetylcholinesterase (AChE), a phenomenon which has its counterpart in the qualitative physiological properties of these ions.[1,2]

Using AChE, Bergmann and Segal[3] have reported the 50% inhibition indices (pI_{50}) for the series methyl- to *n*-heptyltrimethylammonium ions and showed that a roughly linear relationship exists between the pI_{50} values and the number of carbon atoms in the alkyl chains. For our purposes, it was essential to evaluate

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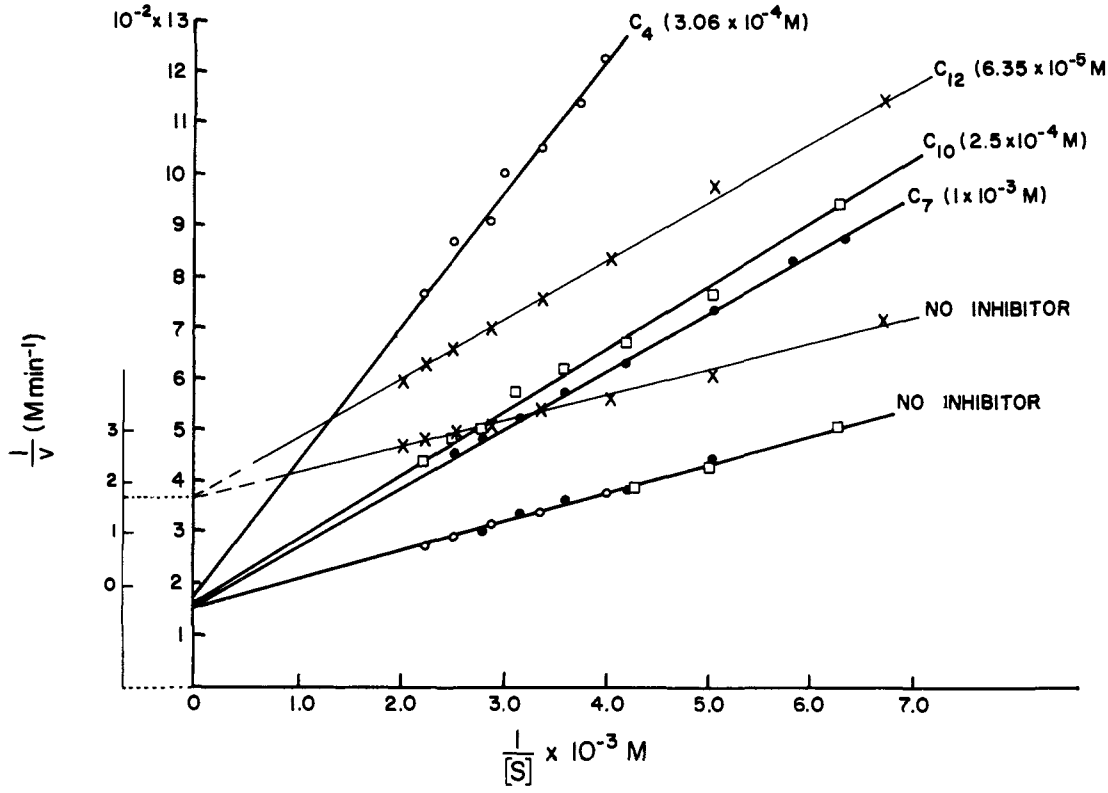

Figure 1. Lineweaver–Burk plots for the binding of butyl-, heptyl-, decyl-, and dodecyltrimethylammonium bromide on AChE. Common intercepts were also obtained with the other members of the series. The standard deviation in the estimation of the initial velocities v was $\pm 6\%$ of the mean. The largely competitive behavior of all the inhibitors is clearly evident.

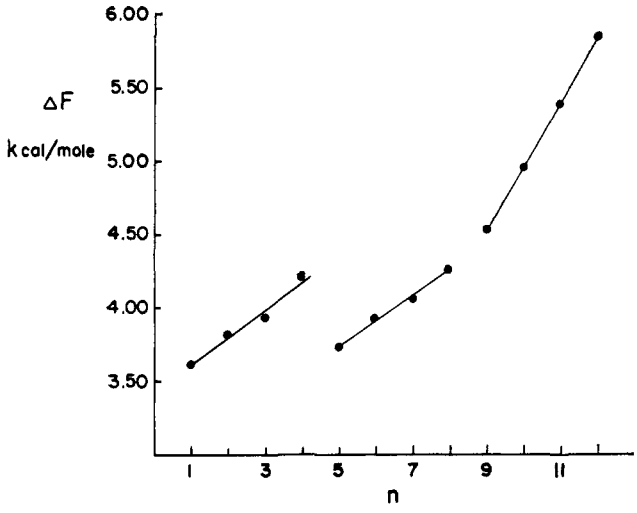

Figure 2. Plot of the free energy of binding at 25° of the $C_nH_{2n+1}N^+(CH_3)_3$ series of AChE inhibitors against n, the number of carbon atoms in the chains. The standard deviation from the mean ΔF values is ± 0.01 kcal. (triplicate determinations).

inhibition constants for the series methyl- to *n*-dodecyltrimethylammonium bromides (C_1 to C_{12}) under conditions such that each substance will inhibit AChE in a largely competitive manner so that there should be no question as to the identity of the active sites participating in addition–complex formation. In order to gain an insight into the factors affecting complex formation, the temperature dependence of the inhibition constants, K_i, was studied at four temperatures, and the separate contributions of enthalpy and entropy to the free energy of binding were calculated from Arrhenius plots in the usual manner.

Bovine erythrocyte AChE from a commercial source was used. Acetylcholine bromide and the inhibitors methyl- to dodecyltrimethylammonium bromide were recrystallized to constant melting point before use. The physical constants agreed with the literature values. All kinetic measurements were carried out at 10, 20, 25, and 30 ± 0.05° with the aid of a pH-Stat. The incubation medium was made 0.1 *M* in NaCl and 0.04 *M* in $MgCl_2$; the initial rates of acetylcholine bromide hydrolysis were measured under a CO_2-free nitrogen atmosphere at pH 7.4 ± 0.05. These evaluations were reproducible to within 6%. The competitive behavior of all the inhibitors was established at 25° using eight different concentrations of substrate while adjusting the inhibitor concentrations so as to depress the initial velocities of hydrolysis by 30–40% of the control value. Some typical results are illustrated in Figure 1 where the standard deviation for each point does not exceed 6% of the mean value. The K_i values obtained from the Lineweaver–Burk plots[4] agreed with the K_i values obtained from the slopes of plots of v_0/v_i against [I], where v_0 is the uninhibited rate, v_i the inhibited rate, and [I] the inhibitor concentration. Three separate runs at 25° and duplicate runs at the other temperatures established that the K_i values are accurate to within 10% (Table I). From the Arrhenius plots which were all linear the enthalpies and entropies of binding were calculated (Table I). A plot of the free energies of binding against n (the number of

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Table I. K_i Values and Thermodynamic Constants for the Binding of $C_nH_{2n+1}N^+(CH_3)_3$ on AChE

n	$K_i^{25°}$; 10^4 M	ΔF,[a] kcal./mole	ΔH, kcal./mole	ΔS, e.u.	Max. cholinergic response,[b] %
1	23.3 ± 0.07	−3.59	−6.60 ± 0.30	−10.1 ± 1.0	100
2	16.0 ± 0.05	−3.81			100
3	13.4 ± 0.05	−3.92	−6.32 ± 0.30	−8.1 ± 1.0	100
4	8.4 ± 0.04	−4.20	−5.22 ± 0.22	−3.4 ± 0.7	100
5	17.4 ± 0.02	−3.76	−5.40 ± 0.20	−5.5 ± 0.7	100
6	13.4 ± 0.05	−3.92			100
7	10.2 ± 0.05	−4.08	−4.49 ± 0.10	−1.4 ± 0.4	60–80[c]
8	6.6 ± 0.15	−4.34	−4.40 ± 0.05	−0.2 ± 0.2	20–40[c]
9	4.8 ± 0.11	−4.53	−4.40 ± 0.05	+0.44 ± 0.17	5–10[c]
10	2.26 ± 0.05	−4.97	−4.26 ± 0.10	+2.4 ± 0.4	0[d]
11	1.16 ± 0.05	−5.37			0[d]
12	0.52 ± 0.07	−5.85	−2.75 ± 0.10	+10.4 ± 0.5	0[d]

[a] Reproducibility is ±0.01. [b] Data from ref. 1 and E. J. Ariëns, "Molecular Pharmacology," Vol. I, Academic Press Inc., New York, N. Y., 1964, pp. 164, 295. [c] Partial stimulants and partial antagonists. [d] Antagonists.

carbon atoms in the side chain of the inhibitors) is shown in Figure 2.

The results shown in Figure 2 are in partial disagreement with the earlier report of Bergmann and Segal[3] in that a significant break in the free-energy relationships with n occurs at C_5. However, the slopes from C_1 to C_4 and C_5 to C_8 are almost identical. More significant is the break at C_9, where a third linear relationship of markedly different slope and extending to C_{12} occurs. Each CH_2 group in the C_9 to C_{12} series has a significantly greater affinity for the enzyme than the CH_2 groups of the lower homologs. This difference is not attributable to a shift from competitive to noncompetitive inhibition (or *vice versa*) (Figure 1). It appeared probable that the higher homologs C_9 to C_{12} interact with the enzyme active surface by a different mechanism (but nevertheless competitive) than the shorter chain members. That this is the case becomes evident when the enthalpies and entropies of binding are examined (Table I). Whereas the homologs from C_1 to C_7 (including C_2 and C_6 by inference) have *negative* entropies of binding (the C_8 member producing little change in ΔS), the C_9 to C_{12} (C_{11} included by inference) members have a *positive* entropy of complex formation. The lower enthalpies of binding of the longer chain members is amply compensated by the positive values for their entropies of adsorption on AChE. This chain length dependent transition from negative to positive entropies of complex formation is not attributable to micelle formation with the long-chain members since the concentrations for inhibition are about 100 times lower than the critical micelle concentration of the dodecyl ion.[5] This transition from an ordering ($-\Delta S$) to a disordering effect ($+\Delta S$) upon complex formation with AChE finds a remarkable parallel in the well-documented transition from stimulant to prevalent antagonistic activity with a chain of similar length in the same series of quaternary ions at the level of certain physiological cholinergic receptors.[1,2] This phenomenon is illustrated in Table I (last column). It seems reasonable on that basis to ascribe the stimulant activity of the C_1 to C_7 members and the antagonistic properties of the C_9 to C_{12} homologs to the induction of ordering and disordering effects, respectively, at the receptor protein level. The above findings provide a rational physicochemical basis for the concept of a dualism in drug-induced perturbations of physiological receptors[6] and point to a biophysical link between the AChE active surface and the binding sites of certain cholinergic receptors. It appears to be the first time that drug-induced stimulation or blockade can be rationalized in basic physicochemical terms. The significance of these findings in relation to receptor theory will be discussed elsewhere.

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On Arylboron Free Radicals

Sir:

In a recent article Leffler, Doland, and Tanigaki[1] reported the e.s.r. spectra of the reaction product of dimesitylboron fluoride with sodium–potassium alloy and its complex with pyridine. They found that the first product gives the same e.s.r. spectrum as trimesitylboron (TMB) after prolonged reaction with Na–Hg. They state that TMB treated with Na–Hg in THF first gives a broad four-line spectrum with a splitting of 8 gauss, and that after prolonged reduction a new spectrum appears with more hyperfine structure and a boron hyperfine splitting of 10 gauss. Their conclusions are that dimesitylboron fluoride over the Na–K alloy gives radical **1**, that addition of pyridine to this radical gives radical **2**, and that the TMB negative ion **3** (which gives the four-line spectrum) decomposes to **1** after prolonged reduction.

$$\underset{\mathbf{1}}{Mes_2B\cdot} \qquad \underset{\mathbf{2}}{Mes_2\dot{B} \rightarrow Pyr} \qquad \underset{\mathbf{3}}{Mes_3B\cdot}$$

Previous studies of TMB[2,3] and recent experiments performed in our laboratory do not agree with their conclusions.

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