completely in disagreement with those obtained by Rossini and co-authors[7] as shown in Table X; while the experimental values of Pines, Kvetinskas, Kassel and Ipatieff are in excellent agreement. The reason for this difference is unknown and further work will be necessary to resolve this discrepancy.

TABLE IX

EQUILIBRIUM OF PENTANES AT 160°

Experiment	1	2	3
Charge			
Mole % isopentane	77.3	82.5	80.6
Mole % *n*-pentane	22.7	17.5	19.4
H_2 pressure at room temp.	750	750	0
Benzene inhibitor, %	1.0	1.0	3.0
Reaction time, minutes	65	65	65
Temperature, °C.	161	163	157
Reactor pressure, p. s. i.	1455	1485	495
V/L Ratio at reaction conditions	Liquid	Liquid	0.1
Products			
Mole % decomposition	1.1	2.3	44.6
Mole % based on pentanes			
Liquid phase: Isopentane	76.9	77.4	78.4
Liquid phase: *n*-Pentane	23.1	22.6	21.5
Vapor phase: Isopentane	78.3	78.8	79.9
Vapor phase: *n*-Pentane	21.7	21.2	20.1

TABLE X

COMPARISON OF HEAT OF ISOMERIZATION AND ENTROPY OF ISOMERIZATION DATA FOR PENTANES

	ΔH cal./mole	ΔS e. u.
Rossini and co-authors	−1930	−1.32
This paper	−3600	−5.87
Pines and co-authors	−1861	−1.30

Experiment 3, Table IX, was made in the absence of hydrogen pressure to determine its effect on the pentane equilibrium. There was approximately a 1% increase in isopentane composition indicating no significant effect due to hydrogen pressure. Likewise side reactions to the extent of 44.6% had no important effect on the chemical equilibrium.

Acknowledgment.—The authors are indebted to the numerous members of the Standard Oil Company (Indiana) Research Laboratories who contributed their advice and services and especially to Dr. W. E. Kuentzel for design of the reactor and Mr. J. E. Swearingen for help in calculation of the fugacities.

Summary

1. The experimental equilibria among the isomeric hexanes were determined in the range 21 to 204° using activated aluminum halide catalysts on a 67° end-point light naphtha stock containing both pentanes and hexanes.

2. The results for hexanes agree with the equilibrium values calculated from the heat of combustion and entropy for *n*-hexane and 2,3-dimethylbutane but there is considerable discrepancy for 2,2-dimethylbutane and the methylpentanes. However, there is substantial agreement in the ratio of 2-methylpentane to 3-methylpentane.

3. The experimental equilibria for the pentanes were also determined and show a higher temperature coefficient than that obtained by Pines, Kvetinskas, Kassel and Ipatieff.

4. Hydrogen and extensive side reactions were found not to have any appreciable effect on the equilibria.

WHITING, INDIANA RECEIVED[13] SEPTEMBER 27, 1948

(13) Original manuscript received July 8, 1946.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Studies in Stereochemistry. XIV. Reaction of Triethylamine and Quinuclidine with Alkyl Halides; Steric Effects in Displacement Reactions[1]

BY HERBERT C. BROWN[2] AND NELSON R. ELDRED[3]

In previous papers of this series, it has been demonstrated that the stability of addition compounds of the type $R_3N:BR'_3$ invariably decreases as the steric requirements of either R or R′ increase. Information gained regarding the effect of structure on the stability of addition compounds should aid in understanding the factors involved in typical displacement reactions.[4]

(1) This paper is based upon a dissertation submitted by Nelson R. Eldred in August, 1946, to the Graduate School of Wayne University in partial fulfillment of the requirements for the degree of Master of Science.

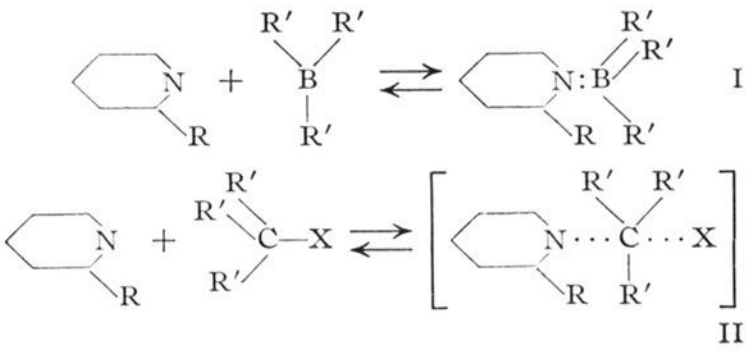
(2) Present address: Department of Chemistry, Purdue University.

(3) Present address: Department of Chemistry, Pennsylvania State College.

(4) Brown and Barbaras, THIS JOURNAL, 69, 1137 (1947).

Consider the reaction of amines with alkyl halides. It is well established that an increase in the steric requirements of the amine increases the steric strain in the addition compound (I) and results in a marked decrease in stability. Similarly, an increase in the steric requirements of the amine should result in a marked increase in steric strain in the activated complex (II) formed from the amine and alkyl halide. In the first instance, a decrease in the heat of dissociation is observed, corresponding in magnitude to the increase in steric strain; in the second, an increase in the energy of activation should be noted, corresponding to the increased strain in the activated complex.

Increase in the steric requirements of the groups



I

II

R′ attached to either boron in the addition compound or to carbon in the activated complex should also bring about increase in steric strain with similar results.[5]

Considerations of this kind lead to the following generalizations for the effects of changing steric requirements on the reactions of amines with alkyl halides. 1. Of two amines of equal base strengths but different steric requirements, the base with the lower steric requirements will react more rapidly with a given alkyl halide. Moreover, the reactions of the base with the greater steric requirements will involve higher activation energies, corresponding to the increase in steric strain. 2. The greater the steric requirements of the alkyl halide, the slower should be the reaction with a given amine. Thus the rate should decrease in the order MeX > EtX > *i*-PrX > *t*-BuX, and the energies of activation should increase in the opposite order. 3. The lower the steric requirements of the alkyl halide, the smaller should be the effect of differences in the steric requirements of the bases. Conversely, the greater the steric requirements of the alkyl halide, the larger should be the effect of differences in the steric requirements of the bases.

To test the validity of these generalizations, as well as the validity of the proposed similarity between the formation of addition compounds and the formation of activated complex, it was decided to measure and compare the rates of reactions of a series of alkyl halides with two amines of similar base strength but with markedly different steric requirements.

In the preceding paper of this series evidence was advanced to support the conclusion that the steric requirements of triethylamine (Fig. 1) are markedly greater than those of quinuclidine (Fig. 2).[6] Although the strengths of the two bases in aqueous solution are not markedly different, their addition compounds with trimethylboron show an amazing difference in stability. Quinuclidine forms an exceedingly stable addition compound, with heat of dissociation of 19.94 kcal., whereas the corresponding addition compound of triethylamine exists only at low temperatures and its heat of dissociation has been estimated to be as low as 10 kcal.[7]

Quinuclidine and triethylamine appeared to fill all the requirements for the two test amines. Accordingly, a study was made of the rate of reaction of quinuclidine with methyl, ethyl and isopropyl iodides and of triethylamine with ethyl iodide. The results, together with other rate data available in the literature, permit a thorough test of the proposed generalizations.

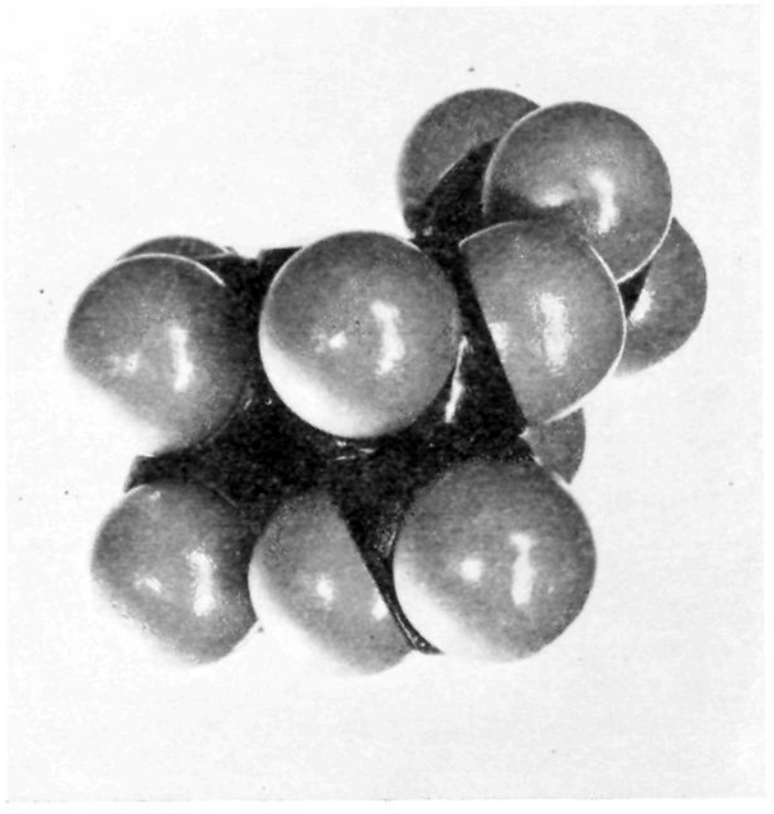


Fig. 1.—Molecular model of triethylamine (note hindered approach to nitrogen atom).

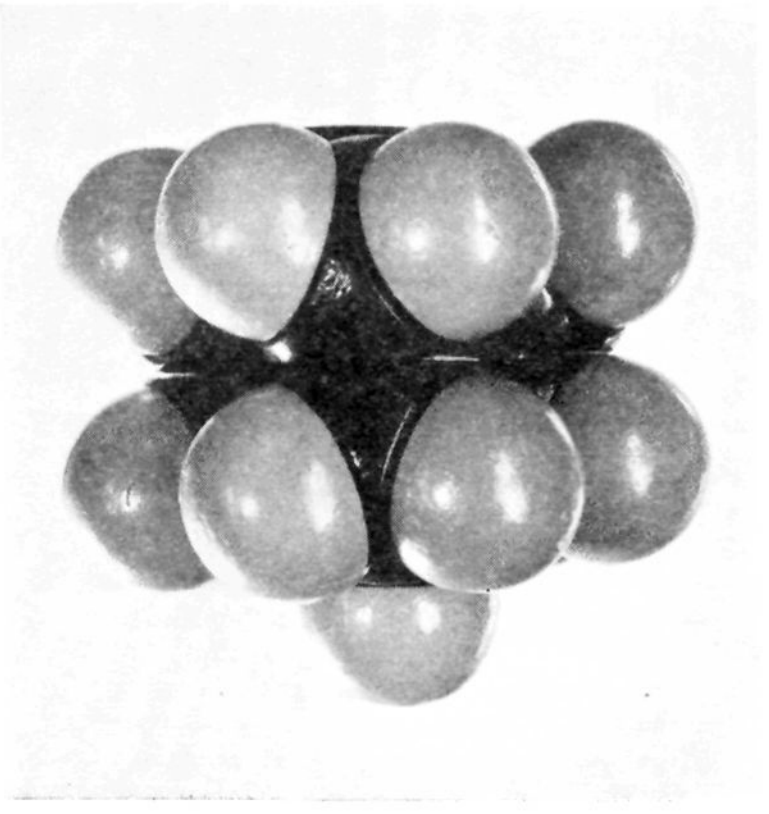


Fig. 2.—Molecular model of quinuclidine (note free approach to nitrogen atom).

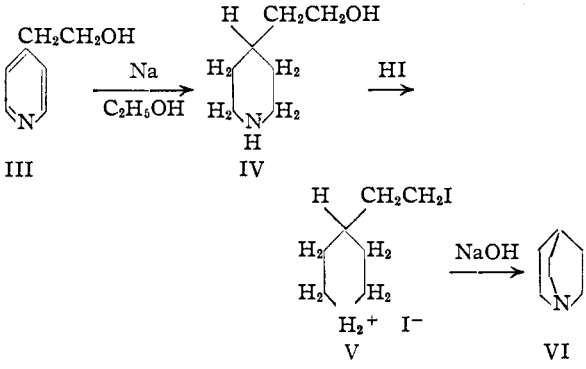
(5) In this discussion it has been assumed that the changes in the groups R and R′ would be of such a nature as not to alter significantly the inherent donor and acceptor properties of the base and acid, respectively.

(6) Brown and Sujishi, THIS JOURNAL, **70,** 2878 (1948).

(7) Brown and Taylor, *ibid.*, **69,** 1332 (1947).

Results

Quinuclidine (VI) was conveniently synthesized by the following series of reactions,[8] starting with the commercially available 4-pyridineëthanol (III).



The rates of reaction with the alkyl iodides were determined in nitrobenzene solution. The reaction was followed by estimation of the free amine by titration.

The results, together with earlier data from the literature for triethylamine and pyridine are listed in Table I.

TABLE I

RATE DATA FOR THE REACTION OF TERTIARY AMINES WITH ALKYL IODIDES IN NITROBENZENE SOLUTION

Amine	Alkyl iodide	$k_{25°}$ (liters mole^{-1} sec.$^{-1}$)	$E_{exp.}$, kcal.	log PZ
Triethylamine	Methyl[a]	3.29×10^{-2}	9.7	5.65
	Ethyl[b]	1.92×10^{-4}	12.5	5.44
	Isopropyl[a]	1.13×10^{-6}	16.0	5.86
Quinuclidine	Methyl[b]	1.88	9.5	(7.2)[c]
	Ethyl[b]	4.87×10^{-2}	10.9	6.68
	Isopropyl[b]	7.97×10^{-4}	13.6	6.86
Pyridine	Methyl[a]	3.43×10^{-4}	13.6	6.50
	Isopropyl[a]	1.55×10^{-6}	16.7	6.43

[a] Laidler and Hinshelwood, *J. Chem. Soc.*, 852 (1938). [b] Present investigation. [c] Reaction was so very rapid that the possibility of considerable error exists, in spite of the satisfactory precision of the experimental data.

Examination of the data from the point of view of the proposed generalizations on the effects of steric requirements is quite interesting.[9] 1. Comparing quinuclidine and triethylamine, two bases of approximately equal base strength but with markedly different steric requirements, it is noted that quinuclidine, the amine of lower steric requirements, reacts considerably faster than triethylamine with each of the three alkyl iodides investigated. At 25°, k_Q/k_T = 57, 254 and 705 for methyl, ethyl, and isopropyl iodides, respectively.[10] Again, the reaction of quinuclidine with a given alkyl halide involves the lower energy of activation in each case. 2. The rates show a marked decrease from methyl to ethyl to isopropyl iodide and the energies of activation show a corresponding increase. This accords with the predicted behavior based on the effect of increasing steric requirements of the alkyl groups. 3. Finally, it is observed that the greater the steric requirements of the alkyl halide, the greater are the effects of differences in the steric requirements of the bases. Thus, in the case of methyl iodide, where the steric requirements of the methyl group are relatively small, the differences in the rate constant and activation energy with the two bases, quinuclidine and triethylamine, are relatively small: at 25°, k_Q/k_T = 57; $E_T - E_Q$ = 0.2 kcal. However, as the steric requirements of the alkyl group become larger, these differences become greater. For ethyl iodide at 25° k_Q/k_T = 254 and $E_T - E_Q$ = 1.6 kcal., and for isopropyl iodide k_Q/k_T = 705 and $E_T - E_Q$ = 2.4 kcal.

The results clearly support the proposed generalizations on the effect of steric requirements on the rate of a typical displacement reaction of the type under consideration.

These generalizations were primarily based upon a postulated similarity between the formation of addition compounds and the formation of activated complexes in displacement reactions. Experimental verification for the proposed generalizations also lends credence to the proposed relationship, and indicates that the study of addition compounds may contribute considerably to a better understanding of the effect of structure on the rates of displacement reactions.

One further point of interest should be mentioned. Hammett[11] has pointed out that reactions involving meta and para derivatives of benzene involve constant entropies of activation and lend themselves particularly well to quantitative treatment. Apparently, the rigidity of the benzene ring and the location of the substituent far from the reaction center causes the substituent to have little effect upon the entropy of activation. On the other hand, structural changes in aliphatic compounds produce marked changes in the entropy of activation and the effect of substituents in such compounds cannot be simply treated.

It is, therefore, of interest that the data in Table I indicate that with the exception of methyl iodide (see note [c] in Table I), the entropies of activation

(8) Meisenheimer, Neresheimer and Schneider, *Ann.*, **420**, 190 (1920).

(9) It has long been recognized that in the absence of important steric effects, the stronger of two amine bases should react faster with a given alkyl halide. This generalization results from the Polanyi picture of the displacement reaction [Evans and Polanyi, *Trans. Faraday Soc.*, **34**, 11 (1938)]. Although this generalization is well established and accepted, it is of interest that the experimental results are in complete agreement. Thus, quinuclidine and pyridine are two bases of similar (low) steric requirements, but of markedly different base strength. In accordance with prediction, quinuclidine, the stronger of the two bases, reacts much more rapidly with either methyl iodide or isopropyl iodide. Moreover, the reactions with quinuclidine involve lower energies of activation.

(10) In the ensuing discussion the symbols, k_Q, k_P, and k_T will be used to refer to the specific rate constants for quinuclidine, pyridine and triethylamine, respectively, and the symbols E_Q, E_P, E_T to the energy of activation for the amine indicated.

(11) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, New York, N. Y., 1940, pp. 121-124

for the reactions of quinuclidine and alkyl iodides are in close agreement with the corresponding quantities for the pyridine reactions. This suggests that the cage system of quinuclidine gives the molecule sufficient rigidity that in its effect upon the entropy factor quinuclidine resembles more closely the rigid aromatic ring systems than it does the flexible, open-chain aliphatic compounds.

More detailed studies of the reactions of quinuclidine and pyridine (and their derivatives) with alkyl halides are underway. It is hoped that more extensive and more accurate data from these investigations will permit a detailed analysis of this interesting problem.

Discussion

It has long been recognized that the rates of reactions involving nucleophilic displacements on carbon vary with the alkyl group in the order, methyl > ethyl > isopropyl > *t*-butyl.[12] Two different interpretations of the observed order have been advanced. Meer and Polanyi[13] attributed the observed order to steric hindrance effects. However, this interpretation was not widely adopted by workers in the field. Prevailing opinion has appeared to favor the idea that the decrease in rate in these reactions with increasing branching was primarily a polar effect. The increasing number of alkyl groups attached to the reaction center was considered to increase the electron density at that center (+I effect of alkyl groups) and thereby hinder the approach of the nucleophilic reagent.

Typical of the viewpoint is the discussion by Hinshelwood, Laidler and Timm.[14] These workers attribute the pronounced rise in activation energy observed in the reactions of amines with methyl and isopropyl iodides to "the diminished positive charge on the carbon atom which attracts the approaching base." The increase in activation energy in passing from methyl iodide to isopropyl iodide is considerably larger for the stronger base, triethylamine, than for the weaker base, pyridine ($E_{\text{T-}i\text{-PrI}} - E_{\text{T-MeI}} = 6.3$ kcal.; $E_{\text{P-}i\text{-PrI}} - E_{\text{P-MeI}} = 3.1$ kcal.). The authors consider these differences in the energies of activation to be especially significant and to offer strong support for the polar interpretation they favor.

The argument follows. It is considered that the nitrogen atom of the stronger base, triethylamine, will carry a considerably higher negative charge than the nitrogen atom of the weaker base, pyridine. With the increased number of methyl groups attached to the reaction center of the alkyl halide, the increased negative charge at that center (or decreased positive charge) will hinder the approach of the amine, but the hindrance should be particularly effective for the amine with the higher negative charge, namely, triethylamine. The reaction will, therefore, require a considerable increase in the energy of activation to overcome the large electrostatic repulsion forces.

This interpretation satisfactorily accounts for the observed differences in the behavior of triethylamine and pyridine in terms of their marked difference in base strength. However, it cannot be extended to cover the behavior of quinuclidine. The present study reveals that, in these displacement reactions, this base, one comparable in strength to triethylamine and much stronger than pyridine, is much more like pyridine in its behavior than like triethylamine. For example, the difference in the energies of activation for the reactions of quinuclidine with isopropyl and methyl iodides ($E_{\text{Q-}i\text{-PrI}} - E_{\text{Q-MeI}}$) is 4.1 kcal., whereas the corresponding quantities for reactions involving pyridine and triethylamine are 3.1 and 6.3 kcal., respectively.

The electrostatic hypothesis favored by Hinshelwood, Laidler and Timm does not appear capable of accounting for the behavior of quinuclidine. Moreover, the data offer no support for the widely-held view that the decreased susceptibility of isopropyl and *t*-butyl halides to attack by nucleophilic reagents is primarily a result of increased electron accession at the reaction center. Until definite evidence of the importance of such electron accession on the rate of nucleophilic displacements is available, it appears preferable to interpret the results of displacement reactions of alkyl halides primarily in terms of the nucleophilic characteristics of the displacing agent and the steric requirements of the reagents, as outlined in the generalizations presented in this paper.

It is hoped that an extensive investigation of the problem, now underway at Purdue University and supported by the Office of Naval Research, will aid in clarifying the situation.

Experimental Part

Materials.—With the exception of quinuclidine, the chemicals were commercially available products, which were purified by standard procedures. The nitrobenzene was purified by partially freezing the material which melted in the range from 5.1 to 5.7°, with most of the material melting from 5.5 to 5.7°. A total of 4 l. was prepared and used to make up all solutions.

Quinuclidine.—Quinuclidine was originally prepared by Löffler and Stietzel[15] in impure form and later prepared in pure form by Meisenheimer and his co-workers.[8] These authors isolated 4-picoline from commercial picoline in poor yield. By treatment with formaldehyde the 4-picoline was converted to 4-pyridineëthanol in yield of but 1.5%. Although the yields in subsequent stages of the synthesis (III–VI) were satisfactory, this minute yield in the initial stages deterred other investigators from following the Löffler–Meisenheimer synthesis. Other syntheses were, therefore, developed and utilized.[16]

The commercial availability of 4-pyridineëthanol from the Reilly Tar and Chemical Corporation led to the selection of the Löffler–Meisenheimer synthesis as most convenient.

(12) Evans, "The Reactions of Alkyl Halides in Solution," The Manchester University Press, Manchester, 1946, pp. 1-2.

(13) Meer and Polanyi, *Z. physik. Chem.*, **B19**, 164 (1932).

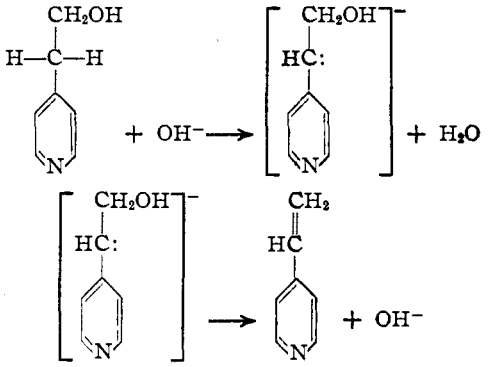
(14) Hinshelwood, Laidler and Timm, *J. Chem. Soc.*, 848 (1938).

(15) Löffler and Stietzel, *Ber.*, **42**, 124 (1909).

(16) Clemo and Metcalfe, *J. Chem. Soc.*, 1989 (1937); Prelog *et al.*, *Ann.*, **525**, 292 (1936); **532**, 69 (1937); **535**, 37 (1938); **545**, 229 (1940).

The commercial product was purified by distillation through a Vigreux column. The fraction which distilled at 146–147° at 8 mm. was utilized. Various fractions gave values for the refractive index of $n^{25}D$ 1.5388 ± 0.0003. The product was very viscous and exceedingly hygroscopic—considerable difficulty was encountered in obtaining satisfactory melting or freezing points. However, a number of fractions, previously frozen, melted in the range from 13.1 to 13.3°.

Attempts to dry the compound by contacting the material with solid potassium hydroxide led to the conversion of the 4-pyridineëthanol to 4-vinylpyridine (b. p. 54° at 7 mm.; $n^{25}D$ 1.5490–1.5495). The 4-pyridineëthanol apparently undergoes a base-catalyzed elimination reaction with remarkable ease. It is probable that the attack is on the active hydrogen atoms, alpha to the pyridine ring.



Several attempts were made to hydrogenate the 4-pyridineëthanol in an Aminco hydrogenator over Raney nickel or U. O. P. nickel catalyst. However, no evidence of hydrogen absorption was observed at 2000 lb. pressure and temperatures as high as 200°. Instability of the compound prevented investigation of higher temperatures. The compound was successfully hydrogenated with sodium and alcohol. The piperidine derivative (IV) was isolated in 75% yield, b. p. 148–153° at 18 mm., $n^{25}D$ 1.4980.

The piperidine derivative was converted into 4-(β-iodoethyl)-piperidine hydroiodide (V) by treatment with concentrated hydriodic acid and red phosphorus. The product, after purification by recrystallization, was obtained in 50% yield. Further recrystallized from a mixture of 60 parts ethyl acetate to 20 parts ethanol it was obtained as nearly colorless crystals which melted at 198–199° as compared to the 190–191° value reported by Meisenheimer.

Previous workers[8] had cyclized the hydroiodide by addition of sodium hydroxide to a dilute solution of the salt in water. The free quinuclidine was distilled from the reaction with water and recovered as the picrate. The picrate was then transformed into the free base. This procedure for isolating the quinuclidine was found to be tedious and the yields unsatisfactory. It was, therefore, discarded in favor of isolation of the base as the hydrochloride. The procedure follows.

The aqueous distillate containing quinuclidine was collected in a flask containing 15 ml. of 15% hydrochloric acid (excess). The distillation was stopped as soon as the distillate was no longer basic to litmus, and the distillate evaporated to dryness. The yield of crude salt was 5.1 g., 75% of the theoretical from the 4-(β-iodoethyl)-piperidine hydroiodide. The hydrochloride was recrystallized from 50 ml. of *n*-butyl alcohol. It was obtained in the form of brilliant white crystals, which melted with decomposition and sublimation at 369–374° in a sealed tube. The salt was analyzed for chloride ion: Calcd. for $C_7H_{14}NCl$: Cl, 24.01. Found: Cl, 24.04.

The free base was prepared by running a concentrated aqueous solution of the hydrochloride into an evacuated flask containing a large excess of potassium hydroxide pellets. The free base was distilled under high vacuum at room temperature from the flask into a U-tube fitted with stopcocks at the entrance and exit. Traces of water which co-distilled could be removed by treatment with sodium or by fractional distillation through a series of such U-tubes, the water being removed as the more volatile component. The yields were practically quantitative. The quinuclidine was obtained as a transparent, glassy solid, which, when dry, crumbled easily.

Vapor pressure data for quinuclidine are reported in an earlier paper.[6]

Kinetic Studies.—Equimolar solutions of amine and alkyl iodides in nitrobenzene were prepared. Usually the concentration of the stock solutions was 0.200 *N*, so that on mixing equal volumes of the two stock solutions the concentration would be 0.100 *N*. For the faster reactions, where it was desirable to have less concentrated solutions, the stock solutions were diluted with the solvent before they were mixed. For temperatures other than 25°, the concentrations of the reactants were corrected for expansion or contraction of the solvent.

The reactants, brought to constant temperature, were placed in a Kjeldahl flask closed with a tightly fitting stopper. After suitable intervals, 5-ml. samples were withdrawn and run into standard *N*/50 hydrochloric acid and the excess acid was back-titrated with *N*/50 barium hydroxide, using methyl red as the indicator.

The rate constants were then calculated using the expression for a second-order reaction in which the two components are present in equal concentrations

$$k = \frac{1}{t} \times \frac{x}{a(a - x)}$$

where t is the time in seconds, x is the moles per liter of amine or alkyl iodide reacted at time t, and a is the initial concentration of amine and alkyl iodide in moles per liter.

The energy of activation for each reaction and the PZ term of the equation

$$k = PZe^{-E/RT}$$

were calculated from the rate constants determined at three to four temperatures, using the method of least squares.

In order to test the procedure, a study was made of the reaction of triethylamine and methyl iodide at 25°. The results are reported in Table II. The value of the rate constant, 0.0329 at 25°, is in satisfactory agreement with the value 0.03085 at 24.8° reported by Laidler and Hinshelwood.[17]

TABLE II

REACTION RATE DATA FOR TRIETHYLAMINE AND METHYI IODIDE IN NITROBENZENE AT 25°

Time, sec.	x (a = 0.0198)	k (liters mole^{-1} sec.$^{-1}$)
1200	0.00876	0.0334
1800	.01066	.0327
2400	.01208	.0329
3600	.01392	.0332
4500	.01476	.0328
5400	.01538	.0325
	Average k =	0.0329

Duplicate determination, average k = 0.0329

The small quantity of quinuclidine available for these studies made it necessary to limit the number of individual samples taken in the course of a determination to 3 or 4. The precision of the data for quinuclidine are, therefore, somewhat

(17) Laidler and Hinshelwood, *J. Chem. Soc.*, 852 (1938).

lower than those reported in Table II for triethylamine and methyl iodide and similar data obtained for triethylamine and ethyl iodide. Moreover, the reaction of quinuclidine with methyl iodide is so rapid (it is essentially complete in four minutes at room temperature with reagents present in as low concentrations as 0.0132 mole per liter), that in this reaction especially the results are subject to considerable uncertainty. It is estimated that the errors in the energies of activation may be as large as 500 cal.

The rate constants obtained are listed in Table III.

TABLE III

RATE CONSTANTS FOR THE REACTION OF TRIETHYLAMINE AND QUINUCLIDINE WITH ALKYL IODIDES

Amine	Alkyl iodide	T, °C.	k (liters mole^{-1} sec.$^{-1}$)
Triethylamine	Methyl	25.0	3.29×10^{-2}
Triethylamine	Ethyl	25.0	1.92×10^{-4}
		35.0	3.81×10^{-4}
		45.0	7.24×10^{-4}
Quinuclidine	Methyl	5.5	0.610
		15.0	1.06
		25.0	1.88
Quinuclidine	Ethyl	5.5	1.27×10^{-2}
		15.0	2.55×10^{-2}
		25.0	4.87×10^{-2}
		35.0	8.34×10^{-2}
Quinuclidine	Isopropyl	25.0	7.97×10^{-4}
		35.0	17.2×10^{-4}
		45.0	31.6×10^{-4}

Values for the energies of activation and log PZ calculated from these values for the specific rate constants are listed in Table I.

Acknowledgment.—The authors wish to acknowledge their debt to Mr. Arno Cahn of the Department of Chemistry of Purdue University for his valuable assistance in checking the calculations of the rate constants and in applying the least squares procedure to the estimation of the energies of activation.

Summary

1. Quinuclidine has been prepared in good yield starting with the commercially available 4-pyridineëthanol. Quinuclidine hydrochloride has been prepared and characterized.

2. Kinetic studies have been made of the reaction of triethylamine with ethyl iodide and of quinuclidine with methyl, ethyl and isopropyl iodide in nitrobenzene solution at several temperatures.

3. A number of generalizations are proposed for the effects of changing steric requirements on displacement reactions. These generalizations, based largely on analogy with the effect of steric requirements on the stability of addition compounds, permit a simple interpretation of the experimental data. The results do not support previous interpretations based primarily on postulated polar effects of structural changes in alkyl groups.

LAFAYETTE, IND. RECEIVED JULY 8, 1948

[CONTRIBUTION FROM CHEMISTRY DEPARTMENT, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Monolayers of Pepsin and of Insulin

BY HECTOR A. DIEU[1] AND HENRY B. BULL

Monolayers of egg albumin and of β-lactoglobulin have been investigated at surface pressures below one dyne per centimeter.[2] The equation represents the relation between the area A and the film pressure F for monolayers of these proteins in the low pressure region. When FA is plotted against F, the slope of the line (α) yields the gaseous area of the film and at 25° the intercept β is equal to 24.6×10^2/mol. wt. where mol. wt. is the number average molecular weight of the film molecules.

$$FA = \alpha F + \beta \qquad (1)$$

It has been found that spread films of pepsin and of insulin obey eqn. 1, and it has thus been possible to calculate the film molecular weights as well as the gaseous area of these proteins.

Experimental

The pepsin was prepared according to Northrop,[3] and its activity was found to be 0.19 Hb. U. per mg. of nitrogen. The pepsin solutions were dialyzed against 0.2 M acetate buffer at pH 5.2 before use. The pepsin concentration was determined by micro-Kjeldahl using the factor indicated by Northrop.[3]

We are grateful to Armour and Company for a supply of crystallin insulin. Before use the insulin was dialyzed against a buffer at pH 3.5 for seventy-two hours at 0°. The concentration of insulin was determined by micro-Kjeldahl.

A Wilhelmy balance has been used to register the film pressures. The wettability of the slides has been improved by the addition of glycerol to the 5% ammonium sulfate solution to the extent of 2% by volume. It is necessary to exercise care in the preparation of the substrate solutions, and the glycerol was exhaustively extracted with petroleum ether and the ether removed completely before addition to the ammonium sulfate solution. The ammonium sulfate solutions were treated with activated charcoal and the charcoal removed by filtering before the addition of the glycerol. The protein solutions were added to the surface with a Blodgett pipet which delivered 0.085 cc. of solution.

Results

The molecular weights and the gaseous areas of spread films of pepsin and of insulin have been calculated with the aid of eqn. 1. Tables I and II

(1) Research Fellow of the Belgian American Foundation.
(2) Bull, THIS JOURNAL, **67**, 4 (1945); **68**, 745 (1946).
(3) Northrop, *J. Gen. Physiol.*, **30**, 177 (1946).