in dioxane: c 0.130 (410–250 mμ), $[\phi]_{314}$ +4150°, $[\phi]_{266}$ −4930°; ultraviolet in dioxane: ϵ^{max}_{285} 33.

2α-Iodocholestan-3-one (II), (Fig. 2) C.D. in dioxane: c 0.940 (350–285 mμ), c 0.188 (285–265 mμ), c 0.047 (265–255 mμ), $[\theta]_{293}$ +5740; O.R.D. in dioxane: c 0.144 (410–260 mμ), $[\phi]_{315}$ +3520°, $[\phi]_{270}$ −6850°; ultraviolet in dioxane: ϵ^{max}_{255} 725.

3α-Bromocholestan-2-one (III), (Fig. 3) C.D. in dioxane: c 0.547 (365–290 mμ), c 0.105 (290–280 mμ), c 0.021 (280–275 mμ), $[\theta]_{316}$ +17200; O.R.D. in dioxane: c 0.121 (420–260 mμ), $[\phi]_{340}$ +12500°, $[\phi]_{290}$ −12200°; ultraviolet in dioxane: ϵ^{max}_{312} 128.

3α-Iodocholestan-2-one (IV).—A solution of 70 mg. of 3α-bromocholestan-2-one (III)[13] in 5 cc. of acetone and 84 mg. of sodium iodide was heated under reflux for 5.5 hr. After cooling and discharging the iodine color by the addition of sodium thiosulfate solution, the product was precipitated by the addition of water, filtered, washed well with water and dried for 48 hr. at 25° (0.01 mm.); yield 58 mg., m.p. 95–98°, $\lambda^{CS_2}_{max}$ 5.88 μ (inflection at 5.84 μ); C.D. in dioxane: c 0.830 (400–305 mμ), c 0.166 (305–250 mμ), $[\theta]_{325}$ +9830. O.R.D. in dioxane: c 0.083 (410–310 mμ); $[\phi]_{350}$ +7160°; ultraviolet in dioxane: ϵ^{max}_{265} 563.

Anal. Calcd. for $C_{27}H_{45}IO$: C, 63.27; H, 8.85; I, 24.76. Found: C, 64.95; H, 9.02; I, 21.27; Br, 0.0.

Several recrystallizations from methanol provided the analytical specimen (30% over-all yield) of 3α-iodocholestan-2-one (IV), m.p. 118–119°, $\lambda^{CS_2}_{max}$ 5.90 μ (same location in CCl_4); (Fig. 4) C.D. in methanol: c 0.770 (390–295 mμ), c 0.385 (295–240 mμ), $[\theta]_{321}$ +18400, $[\theta]_{255}$ −1970. O.R.D. in methanol: c 0.026 (410–270 mμ), $[\phi]_{346}$ +13800°, $[\phi]_{294}$ −15000°; ultraviolet in methanol: $\epsilon^{shoulder}_{330-310}$ 253–359, ϵ^{max}_{274} 494; C.D. in dioxane: c 0.896 (390–320 mμ), c 0.448 (320–280 mμ), c 0.0896 (280–250 mμ), $[\theta]_{324}$ +16600; O.R.D. in dioxane: c 0.026 (410–270 mμ), $[\phi]_{346}$ +13200°, $[\phi]_{290}$ −10400°; ultraviolet in dioxane: $\epsilon^{shoulder}_{330-310}$ 248–316, ϵ^{max}_{274} 495.

Anal. Found: C, 63.54; H, 8.83; I, 24.79.

Sodium Iodide–Acetone Treatment of 3β-Bromocholestan-2-one.—The exchange reaction of 218 mg. of 3β-bromocholestan-2-one[13] was performed exactly as described above for the 3α-isomer and the total crude product (185 mg.) exhibited the properties: m.p. 97–101°, $\lambda^{CS_2}_{max}$ 5.91 μ (inflection 5.83 μ); C.D. in dioxane: c 1.13 (400–320 mμ), c 0.226 (320–207 mμ), c 0.113 (270–250 mμ), $[\theta]_{323}$ +13000; O.R.D. in dioxane: c 0.113 (420–350 mμ); c 0.0226 (320–285 mμ), c 0.0113 (285–275 mμ), $[\phi]_{347}$ +10450°, $[\phi]_{290}$ −9970°; ultraviolet in dioxane: $\epsilon^{shoulder}_{335-315}$ 140–235, ϵ^{max}_{287} 633.

Anal. Calcd. for $C_{27}H_{45}IO$: C, 63.27; H, 8.85; I, 24.76. Found: C, 65.01; H, 9.02; I, 22.54.

ments were employed in this study: Cary Applied Physics Model 14 Spectrophotometer (ultraviolet absorption); Baird-Atomic/Jouan Dichrographe (circular dichroism); Japan Spectroscopic Manufacturing Company automatically recording spectropolarimeter (optical rotatory dispersion).

6α-Iodocholestan-7-one (V), (Fig. 5) C.D.[24] in dioxane: c 1.595 (350–295 mμ), c 0.399 (295–275 mμ), c 0.100 (275–260 mμ), $[\theta]_{327}$ +120, $[\theta]_{287}$ −3670; O.R.D. in dioxane: c 0.122 (410–295 mμ), c 0.0244 (295–265 mμ), $[\phi]_{315}$ −1680°, $[\phi]_{305}$ −1840°; ultraviolet in dioxane: ϵ^{max}_{255} 740.

6β-Iodocholestan-7-one (VI), (Fig. 6) C.D. in dioxane: c 0.298 (390–280 mμ), c 0.0745 (280–250 mμ), $[\theta]_{323}$ +21700, $[\theta]_{255}$ −6800; O.R.D. in dioxane: c 0.120 (410–275 mμ), $[\phi]_{342}$ +13500°, $[\phi]_{292}$ −20700°; ultraviolet in dioxane: ϵ^{max}_{319} 427, ϵ^{max}_{272} 747.

6α-Bromocholestan-7-one (VII), (Fig. 7) C.D. in dioxane: c 2.45 (325–240 mμ), $[\theta]_{287}$ −3060; O.R.D. in dioxane: c 0.244 (410–260 mμ), c 0.0488 (260–240 mμ), $[\phi]_{310}$ −1530°, $[\phi]_{300}$ −1600°; ultraviolet in dioxane: ϵ^{max}_{280} 32.

6β-Bromocholestan-7-one (VIII), (Fig. 8) C.D. in dioxane: c 0.980 (370–255 mμ), c 0.109 (255–240 mμ), $[\theta]_{313}$ +15850; O.R.D. in dioxane: c 0.098 (410–320 mμ), c 0.012 (320–260 mμ), $[\phi]_{335}$ +9600°, $[\phi]_{285}$ −19000°; ultraviolet in dioxane: ϵ^{max}_{305} 178.

21-Iodo-5α-pregnan-3β-ol-20-one (IX).—A freshly prepared[6] specimen was recrystallized from methanol (m.p. 138–139°) and analyzed prior to the optical and spectral measurements; (Fig. 9) C.D. in dioxane: c 1.080 (380–295 mμ), c 0.216 (295–250 mμ), $[\theta]_{318}$ +12750; O.R.D. in dioxane: c 0.100 (410–275 mμ), $[\phi]_{345}$ +8180°, $[\phi]_{285}$ −10240°; ultraviolet in dioxane: $\epsilon^{shoulder}_{330-310}$ 176–247, ϵ^{max}_{266} 438.

Anal. Calcd. for $C_{21}H_{33}IO_2$: C, 56.76; H, 7.48; I, 28.56. Found: C, 56.15; H, 7.55; I, 28.29.

21-Bromopregnane-3α,17α-diol-20-one (X), (Fig. 10) C.D. in dioxane: c 0.816 (370–265 mμ), c 0.163 (265–250 mμ), $[\theta]_{303}$ +4940; O.R.D. in dioxane: c 0.102 (420–250 mμ), $[\phi]_{322}$ +3000°, $[\phi]_{270}$ −3890°; ultraviolet in dioxane: ϵ^{max}_{295} 83.

21-Iodopregnane-3α,17α-diol-20-one (XI).—A solution of 413 mg. of the above 21-bromide X[21] in 25 cc. of acetone and 300 mg. of sodium iodide was heated under reflux for 30 minutes. After cooling in ice, the product was precipitated by the addition of water and the filtered and washed solid was recrystallized three times from aqueous methanol. The colorless needles (133 mg.) proved to be homogeneous on thin-layer chromatography (benzene–methanol 9:1 on silica gel) and exhibited m.p. 143–146°; (Fig. 11) C.D. in methanol: c 0.705 (390–285 mμ), c 0.282 (285–245 mμ), $[\theta]_{307}$ +6000, $[\theta]_{250}$ −1000; O.R.D. in methanol: c 0.0892 (410–325 mμ), c 0.0446 (325–290 mμ), c 0.0178 (290–240 mμ), $[\phi]_{352}$ +4260°, $[\phi]_{274}$ −4380°; ultraviolet in methanol: ϵ^{max}_{292} 368; C.D. in dioxane: c 1.315 (400–300 mμ), c 0.526 (300–270 mμ) c 0.263 (270–250 mμ), $[\theta]_{315}$ +4780; O.R.D. in dioxane: c 0.1184 (410–320 mμ), c 0.0592 (320–260 mμ), c 0.0237 (260–240 mμ), $[\phi]_{360}$ +3110°, $[\phi]_{280}$ −2930°; ultraviolet in dioxane: $\epsilon^{shoulder}_{330-310}$ 128–183, ϵ^{max}_{263} 403.

Anal. Calcd. for $C_{21}H_{33}IO_3$: C, 54.78; H, 7.22; I, 27.57. Found: C, 55.02; H, 7.42; I, 27.44.

(24) The small maximum at 327 mμ probably indicates the presence of a trace amount of 6β-iodo contaminant.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SIR JOHN CASS COLLEGE, LONDON, ENGLAND]

Kinetic Studies of Hydrogen Exchange in Dialkylanilines. Part I

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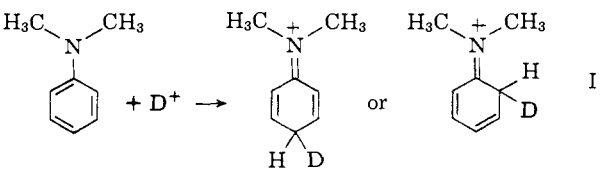
A kinetic study of hydrogen exchange in a series of substituted dialkylanilines indicates that the reaction is a typical electrophilic substitution and that the data conform to the Hammett substituent relationship. Thermodynamic parameters have been obtained which have been considered in relation to the steric inhibition of resonance in the dialkylaniline system.

Introduction

During the past 25 years a number of aromatic hydrogen exchange reactions have been examined in order to determine whether or not the mechanism of exchange conforms to normal aromatic substitution. The early work was carried out with deuterium in which the extent of hydrogen exchange was measured by change in density or infrared spectra.

The acid-catalyzed hydrogen exchange reaction of dimethylaniline with deuterium in aqueous solution was first reported by Ingold, *et al.*,[2] who suggested that exchange occurred by electrophilic substitution of the aromatic hydrogen by the deuterium ion. Kharasch, *et al.*,[3,4] extended the investigation to a study of the exchange in deuterated alcohol. They considered three possible mechanisms but preferred the one based



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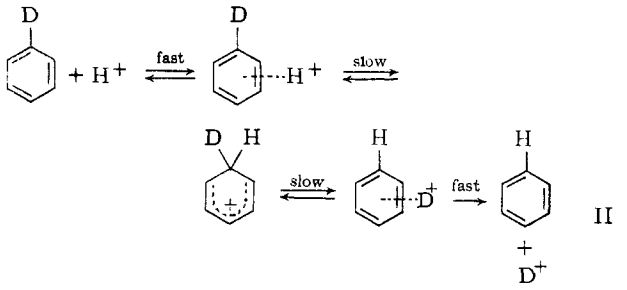
on acid-catalyzed deuterium exchange at nuclear positions of highest electron density, *i.e.*, *ortho* and *para* to the dimethylamino group, giving rise to quinonoid forms of salts as intermediates.

A study of the deuterium exchange reactions of nuclear halogen substituted dimethylanilines by Brown, *et al.*,[5] showed that when chlorine and bromine were substituted in positions *ortho* to the dimethylamino group, hydrogen exchange was negligible, but such interference with exchange was not caused by *meta* and *para* substitution. The *ortho* inhibition was shown to diminish as the substituent became smaller, and *o*-fluorodimethylaniline exhibited some degree of exchange. This was considered to be due to steric effects in the *o*-isomers preventing the nitrogen atom taking up the preferred configuration necessary for its participation in the aromatic resonance system. This interpretation was confirmed by the demonstration of rapid exchange in N-methylindoline where the amino nitrogen is linked to the *o*-carbon atom in such a way that steric inhibition does not occur.

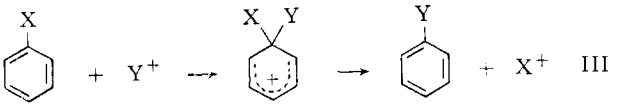
All the early work lacked the rigorous kinetic data needed for the quantitative analysis of the reaction mechanism, but in a series of reports by Gold and Satchell[6] kinetic data were provided for the deuterium exchange in benzene and phenols. Their proposal of a new general mechanism for the aromatic hydrogen exchange reactions drew much attention. By application of the Zucker–Hammett criterion[7] they concluded that the reaction proceeded by a mechanism which involved fast proton transfer equilibrium and slow rate-determining intramolecular transitions (A-1 mechanism II). However, Eaborn and Taylor[8] studying



II

detritiation of benzene and toluene in sulfuric acid solutions were unable to show any significant relationship between exchange rate and acidity function H_0 and concluded that there was no adequate reason to discard the orthodox electrophilic substitution mechanism in favor of the more complex A-1 mechanism. In fact, these authors raised doubts about the usefulness of the Zucker–Hammett criterion for deciding between possible mechanisms.

Further doubts about the Zucker–Hammett criterion[9] led Kresge and Chiang[10] to use a different criterion for detritiation studies of 1,3,5-trimethoxybenzene in aqueous weak acid solutions. They argued that in the four-step (A-1) mechanism the exchange rate would be proportional only to the *p*H of the reaction mixture, *i.e.*, to proceed through specific hydronium ion catalysis. For the orthodox bimolecular mechanism (III), however, the rate would be proportional to the concentration of all acidic species present, *i.e.*, proceed through



III

general acid catalysis. They concluded that the tritium exchange reaction was an acid-catalyzed substitution reaction (A-Se2 type), slow proton transfer to the substrate being the rate-controlling step.

Attempts have been made to resolve the apparent divergences revealed in these various studies by suggesting that the mechanism of exchange may depend upon the nature of the substrate and reaction medium.[11]

The objectives of our studies were to provide kinetic data for acid-catalyzed hydrogen exchanges in dialkylanilines and to re-examine the possible mechanisms of exchange. In particular, studies have been made of a range of substituted dialkylanilines in the same reaction media.

In this series of experiments the rate of hydrogen exchange has been determined by measuring the rate of appearance of tritium in the aromatic substrate using a scintillation counting technique.

Experimental

1. Materials.—N,N-Dimethylaniline (b.p. 191°, lit.[12] b.p. 193° cor. (760 mm.)), N,N-diethylaniline (b.p. 216°, lit.[13] b.p. 216.5° cor. (760 mm.)), N,N-di-*n*-propylaniline (b.p. range 238–242°, lit.[14] 238-241°), *m*-methyl-N,N-dimethylaniline (b.p. 72–74° (5 mm.), lit.[15] b.p. 215° (760 mm.)), *m*-ethoxy-N,N-diethylaniline (b.p. 141–142° (15 mm.), lit.[16] b.p. 145° (14 mm.)), *p*-methyl-N,N-dimethylaniline (b.p. 76.5–77.5° (4 mm.), lit.[17] b.p. 211° (760 mm.)) and *p*-bromo-N,N-dimethylaniline (m.p. 55°, lit.[18] m.p. 55°) were purified from commercially available material by refluxing for 3 hours with 2 gram-equivalents of acetic anhydride followed by fractional distillation at reduced pressure.

N,N-Di-*n*-butylaniline (b.p. 117.5–118.5° (1 mm.), lit.[19] b.p. 270–275° (760 mm.)), *p*-methoxy-N,N-dimethylaniline (m.p. 47°, lit.[20] 47°), *p*-fluoro-N,N-dimethylaniline (m.p. 35°, lit.[20] 35°), *p*-chloro-N,N-dimethylaniline (m.p. 33°, lit.[20] 32.8°), *o*-fluoro-N,N-dimethylaniline (b.p. 47.5° (6 mm.), lit.[5] b.p. 69–70° (16 mm.)), *o*-chloro-N,N-dimethylaniline (b.p. 86–87° (5 mm.), lit.[21] b.p. 207–208.5° (760 mm.)), *o*-methyl-N,N-dimethylaniline (b.p. 71.0–71.5° (15 mm.), lit.[22] b.p. 87° (30 mm.)), *o*-methoxy-N,N-dimethylaniline (b.p. 88.5–89.3 (2 mm.), lit.[22] b.p. 113° (28 mm.)), 2,4-dimethyl-N,N-dimethylaniline (b.p. 72.0° (3 mm.), lit.[23] b.p. 203–205° (760 mm.)), 2,6-dimethyl-N,N-dimethylaniline (b.p. 64.5–65.0° (4 mm.), lit.[24] b.p. 194–199° (760 mm.)) and 3,5-dimethyl-N,N-dimethylaniline (b.p. 80–82° (7 mm.), lit.[25] b.p. 228.5–229° (772 mm.)) were prepared by methylation of the corresponding aniline with dimethyl sulfate following standard procedures.[26] *m*-Nitro-N,N-dimethylaniline (m.p. 60°, lit.[27] m.p. 59–60°) was purified from technical grade material by recrystallization from aqueous alcohol.

The following methiodide derivatives were prepared from the dried tritiated dialkylanilines by standing or refluxing (as necessary) with methyl iodide. The precipitated methiodide was recrystallized to constant melting point from alcohol or alcohol–ether mixtures: N,N-dimethylaniline methiodide (m.p. 228°, lit.[28] m.p. 228°), N,N-diethylaniline methiodide (m.p. 103°,

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lit.[29] m.p. 102°), N,N-di-*n*-propylaniline methiodide (m.p. 156°, lit.[30] m.p. 156°), *m*-methyl-N,N-dimethylaniline methiodide (m.p. 177.5°, lit.[31] 177°), *m*-ethoxy-N,N-diethylaniline methiodide (m.p. 139°; *Anal.* Calcd. for $C_{13}H_{22}NOI$: C, 46.55; H, 6.62. Found: C, 46.70; H, 6.65.), *p*-methyl-N,N-dimethylaniline methiodide (m.p. 218°, lit.[32] 220°), *p*-bromo-N,N-dimethylaniline methiodide (m.p. 188–189° dec., lit.[33] 187–188° dec.), N,N-di-*n*-butylaniline methiodide (m.p. 123–124°; *Anal.* Calcd. for $C_{15}H_{26}NI$: C, 51.85; H, 7.55. Found: C, 50.4; H, 7.50.), *p*-methoxy-N,N-dimethylaniline methiodide (m.p. 234–236° dec.; *Anal.* Calcd. for $C_{10}H_{16}NOI$: C, 40.95; H, 5.50. Found: C, 41.05; H, 5.40.), *p*-fluoro-N,N-dimethylaniline methiodide (m.p. 217–218° dec.; *Anal.* Calcd. for $C_9H_{13}NFI$: C, 38.43; H, 4.66. Found: C, 38.80; H, 4.65.), *p*-chloro-N,N-dimethylaniline methiodide (m.p. 189–190° dec.; *Anal.* Calcd. for $C_9H_{13}NClI$: C, 36.30; H, 4.40. Found: C, 36.10; H, 4.43.), *o*-fluoro-N,N-dimethylaniline methiodide (m.p. 200–201° dec.; *Anal.* Calcd. for $C_9H_{13}NFI$: C, 38.43; H, 4.66. Found: C, 38.80; H, 4.65.), *o*-chloro-N,N-dimethylaniline methiodide (m.p. 148°, lit.[34] 152°), 3,5-dimethyl-N,N-dimethylaniline methiodide (m.p. 219° dec.; *Anal.* Calcd. for $C_{11}H_{18}NI$: C, 45.36; H, 6.18. Found: C, 45.40; H, 6.30.), *o*-methyl-N,N-dimethylaniline methiodide (m.p. 210°, lit.[34] 209°), *o*-methoxy-N,N-dimethylaniline methiodide (m.p. 241–242°, lit.[34] 242°), 2,4-dimethyl-N,N-dimethylaniline methiodide (m.p. 178–179°, lit.[35] 186° cor.).

Solvents required for the preparation of scintillator solutions were purified. **Toluene**: refluxed over sodium metal, followed by double fractional distillation (b.p. 110.5–111.0°, lit.[36] 110–111°); **dioxane**: analytical grade refluxed over anhydrous stannous chloride and fractionally distilled (b.p. 102–103°, lit.[37] 101–103°); **ethanol**: analytical grade refluxed over calcium metal and fractionally distilled (b.p. 78.0–78.5°, lit.[38] 78.3°).

Scintillation grade *p*-terphenyl and 1,4-di-(2,5-phenyloxazolyl)-benzene were used as primary ands econdary solutes for the scintillator solution.

Tritiated Ethanol.—Tritiated water (0.6 ml.) containing 60 millicuries of tritium was added to 100 ml. of redistilled, calcium-dried, absolute alcohol and refluxed for 1 hour over 2 g. of calcium metal. The tritiated alcohol was distilled out through a fractionating column and the fraction in the range 77.5–78.5° was collected.

2. Analytical Procedure. (a) Scintillation Counting of Tritium.—The concentration of tritium incorporated into the dialkylaniline by exchange was determined by measurement of the tritium β-radiation using a liquid scintillation counting technique. Preliminary experiments indicated that counting of the tritiated anilines themselves was unreliable due to traces of contamination by the high specific activity reaction medium and to considerable quenching of the scintillation process by the dialkylaniline. Further work showed that these objections could be entirely overcome by conversion of the tritiated dialkylaniline to the methiodide derivative.

A scintillation counter (manufactured by Panax Equipment Ltd., type SC/LP) was used throughout these studies. The counter uses a single, 11-stage photomultiplier tube (EMI 6097S) mounted in a lead castle providing 1.5 inches of shielding in all directions. The liquid scintillator solution with active sample is contained in a Pyrex glass stoppered bottle of 14-ml. capacity which is optically coupled to the photomultiplier tube with silicone fluid (MS200/20 cs.). An iris diaphragm shutter intervenes between photomultiplier tube and sample chamber whenever the castle door is opened, thus permitting the introduction of samples in ordinary daylight. The whole counter assembly was maintained in a refrigerator kept at 5°; this cooling was adequate to keep the photomultiplier noise contribution down to an acceptable level. The photomultiplier output was fed through a cathode follower to a linear amplifier and discriminator, which operated a Panax type automatic scaler and timer. High voltage for the photomultiplier dynodes was obtained from a high stability power unit.

Optimum counting performance required selection of operating variables to give maximum signal to noise ratio under high voltage plateau conditions. Using visual inspection and a statistical method[39] a final selection of variables gave characteristic curves for sample and background in which the slope of the tritium plateau was 1.9%/100 volts. This is considered to be most satisfactory for a scintillation counter.

Although a number of liquid scintillator formulations were investigated it was found that for this work a scintillator consisting of 3.0 g./l. of *p*-terphenyl and 0.1 g./l. of 1,4-di-(2,5-phenyloxazolyl)-benzene in toluene was most suitable.

The linearity of the counting technique was established by measuring the count rate for a series of accurately weighed quantities of tritiated dialkylanilines. Over the range investigated, a strictly linear relationship between count rate and concentration of methiodide was found, proving that quenching does not occur. The correlation coefficient for this plot was 0.999.

The efficiency of counting under the selected optimum conditions was shown to be about 25% using an internal standard of tritiated hexadecane reference material supplied by the Radiochemical Centre, Amersham.

The satisfactory over-all reproducibility of the counting procedure was established by measurement of counting rate for a large number of accurately weighed samples of tritiated N,N-dimethylaniline methiodide. The coefficient of variation was 0.4%.

For 2,6-dimethyl-N,N-dimethylaniline and *m*-nitro-N,N-dimethylaniline, which do not form methiodide derivatives, it was necessary to count the dialkylaniline itself and in these cases considerable quenching of the scintillation process occurred. By measuring the count-rate for a series of accurately weighed quantities of these tritiated dialkylanilines it was shown that the quenching action followed relationships of the form

$$N = SCe^{-qC} \text{ for 2,6-dimethyl-N,N-dimethylaniline}$$
$$N = SCe^{-qC^{1/2}} \text{ for } m\text{-nitro-N,N-dimethylaniline}$$

where N is the count rate (c.p.s.) at concentration C of dialkylaniline (mg./10 ml.), S is the specific activity in counts sec.$^{-1}$ mg.$^{-1}$ at zero quenching and q is the quenching constant. From the plots of log N/C against C and $C^{1/2}$, respectively, the activity at zero quenching was obtained from the intercept. This method was used for all activity measurements involving the *m*-nitro-N,N-dimethylaniline and 2,6-dimethyl-N,N-dimethylaniline. The experimental data are shown in Table I.

TABLE I

QUENCHING OF 2,6-DIMETHYL-N,N-DIMETHYLANILINE AND *m*-NITRO-N,N-DIMETHYLANILINE

C, mg.	N/C	log N/C	C, mg.	$C^{1/2}$	N/C	log N/C
2,6-Dimethyl-N,N-dimethylaniline[a]			*m*-Nitro-N,N-dimethylaniline[b]			
			0.10	0.316	230.0	2.362
0.94	6.3	0.799	.50	.707	177.0	2.248
1.88	6.2	.792	.80	.894	138.0	2.140
3.76	6.0	.778	1.45	1.204	99.3	1.996
4.70	5.7	.756	2.10	1.449	76.0	1.881
7.05	5.8	.763	3.20	1.789	53.1	1.725
9.40	5.5	.740	3.50	1.871	48.6	1.687
14.10	5.1	.708	4.60	2.145	36.5	1.562
23.50	4.5	.653	5.40	2.342	30.2	1.480
32.90	3.9	.591	6.20	2.490	25.6	1.408

[a] Log $N/C = 0.801 - 0.0064C$. [b] Log $N/C = 2.536 - 0.452C^{1/2}$. Least square slope of: [a] −0.0064, [b] −0.452; standard deviation of slope: [a] 0.0002, [b] 0.002; correlation coefficient r: [a] 0.925, [b] 0 .999; standard deviation in vertical direction σ_y: [a] 0.006, [b] 0.014.

(b) Specific Activity of Tritiated Ethanol.—The specific activity of the tritiated ethanol used in the reaction medium was determined from ten replicate measurements using a 1000:1 dilution technique. The mean value was 7.0×10^6 c.p.s./ml. counted under the same conditions as described above for methiodides.

(c) Kinetic Rate Determination.—The rate of exchange R was determined from the equation[40]

$$R = -\frac{[A][B]}{[A]\beta + [B]\alpha} \times \frac{\ln(1 - F)}{t}$$

where

[A] = concn. of hydrogen ion in the reaction medium (active + inactive)
[B] = concn. of exchangeable hydrogen in the dialkylaniline
F = fraction of exchange occurring in time t

For these experiments the dialkylanilines were initially inactive and the fraction of exchange is given by the ratio S_t/S_∞ where S is the specific activity (c.p.s. mg.$^{-1}$) at times $t = t$ and $t = \infty$

(29) A. Claus and H. Howitz, *Ber.*, **17**, 1326 (1884).
(30) H. O. Jones, *J. Chem. Soc.*, 1407 (1903).
(31) F. J. Smith and E. Jones, "A Scheme of Qualitative Organic Analysis," Blackie and Son Ltd., London, 1951, p. 149.
(32) Beilstein's "Handbuch der Organischen Chemie," 1st ed., Vol. XII, p. 904.
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(37) Reference 36, p. 371.
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TABLE II

EXCHANGE KINETICS FOR *p*-FLUORO-N,N-DIMETHYLANILINE

Temp. (±0.1 °K.)	Reaction period, hr.	Spec. act. c.p.s./10 mg.	−log (1 − F)	Slope[a] × 10⁵	σ[b]	σ_y[c]	r[d]
338.4	24	5.0	0.0060				
	32	7.0	.0084				
	48	10.0	.0121				
	73	15.0	.0182				
	168	37.0	.0462				
	337	70.0	.0921				
	507	101.0	.1401				
	∞	366.5		27.8	0.3	0.0012	0.993
348.0	18	14.0	0.0170				
	42	32.0	.0400				
	66	48.0	.0614				
	90	64.0	.0840				
	162	102.0	.1428				
	210	126.0	.1845				
	259	149.0	.2287				
	350	185.0	.3082				
	∞	364.0		87.0	1.1	0.0034	0.999
356.2	7	11.5	0.0140				
	24	42.0	.0534				
	48	82.0	.1112				
	55	90.0	.1237				
	72	116.0	.1672				
	145	195.0	.3346				
	192	227.0	.4264				
	243	250.0	.5068				
	∞	363.0		213	6	0.0132	0.998
363.8	3	10.0	0.0121				
	8	29.0	.0360				
	24	88.0	.1198				
	32	116.0	.1661				
	48	163.0	.2569				
	56	182.0	.2998				
	72	212.0	.3776				
	80	222.0	.4070				
	∞	365.0		526	8	0.0065	0.999

[a] Least squares slope. [b] Standard deviation of slope. [c] Standard deviation in vertical direction. [d] Correlation coefficient.

and α and β = kinetic isotope effects in the two opposing transfer reactions constituting the exchange equilibrium. These constants were neglected in calculation of R because it was not possible to determine their values from the experimental data. It follows that the value of R will differ from the absolute exchange rate if kinetic isotope effects are operative. The only data on possible isotope effects available at the time of writing are those from the measured exchange numbers which suggests that the *equilibrium* isotope effect for all the compounds studied is similar and very small.

In the calculation of [B] it was assumed for the unsubstituted and *m*-dialkylanilines that each molecule contains three exchangeable hydrogen atoms corresponding to two *o*- and one *p*-positions. For the *o*- and *p*-substituted compounds two exchangeable hydrogen atoms were assumed; justification for these assumptions is discussed below.

In the general procedure adopted for determination of exchange rate the required quantity of purified dialkylaniline (0.025 mole) was weighed into a standard flask and sulfuric acid (d = 1.840, 0.050 mole) added slowly with cooling. Under continuous cooling, the volume was made up to 50 ml. with 1 ml. of tritiated ethyl alcohol and redistilled absolute alcohol (calcium dried) giving 95% alcohol solution by volume; 5-ml. samples were sealed into glass ampoules which were then transferred to a thermostatically controlled bath. For temperatures up to 95° a liquid oil-bath was used, but above this temperature a vapor-bath was found to be more satisfactory. In both cases temperature control was better than ±0.1°.

Ampoules were removed from the bath at timed intervals and immediately cooled in ice-water. The contents were then discharged into excess sodium carbonate (2 N) to liberate the free amine, which was extracted with ether. The methiodide derivative was prepared from the ether solution as described above.

Approximately 10-mg. samples of the methiodide were accurately weighed into a counting bottle followed by 3 ml. of absolute

TABLE III

SUMMARY OF EXPERIMENTAL KINETIC DATA

Aniline	Temp., °K. (±0.1)	log $k + 4$[a]	log PZ	% Reacn. followed
N,N-Dimethyl-	348.1	1.874	15.27	
	353.3	2.101	15.24	
	357.0	2.313	15.28	
	363.1	2.581	15.26	62
N,N-Diethyl-	345.1	0.728	15.66	
	351.8	1.091	15.67	
	358.8	1.462	15.67	
	366.3	1.827	15.66	63
N,N-Di-*n*-propyl-	343.0	1.072	14.22	
	349.0	1.452	14.30	
	355.2	1.706	14.27	
	361.1	1.980	14.27	70
N,N-Di-*n*-butyl-	346.2	1.222	14.02	
	350.6	1.472	14.07	
	356.5	1.725	14.04	
	361.4	1.930	14.02	
	365.5	2.127	14.04	51
p-Methyl-N,N-dimethyl-	339.3	1.154	15.64	
	344.3	1.456	15.67	
	348.7	1.653	15.64	
	356.2	2.031	15.64	
	361.8	2.317	15.65	51
p-Methoxy-N,N-dimethyl-	342.6	0.610	15.43	
	349.7	0.880	15.31	
	356.4	1.239	15.33	
	363.3	1.681	15.43	48
p-Fluoro-N,N-dimethyl-	338.4	0.326	14.47	
	348.0	0.840	14.49	
	356.2	1.232	14.47	
	363.8	1.596	14.48	52
p-Chloro-N,N-dimethyl-	342.6	1.430	12.66	
	349.7	1.669	12.61	
	356.4	1.967	12.63	
	363.3	2.279	12.66	64
p-Bromo-N,N-dimethyl-	337.0	1.113	12.22	
	345.2	1.479	12.23	
	353.5	1.819	12.23	
	363.2	2.204	12.22	55
m-Methyl-N,N-dimethyl-	340.9	2.517	14.86	
	347.3	2.835	14.88	
	355.2	3.182	14.87	
	361.4	3.490	14.90	81
3,5-Dimethyl-N,N-dimethyl-	343.4	3.138	16.55	
	351.1	3.496	16.53	
	357.0	3.801	16.55	99
m-Ethoxy-N,N-diethyl-	332.7	2.454	16.56	
	339.4	2.820	16.52	
	346.7	3.183	16.52	77
m-Nitro-N,N-dimethyl- (primary reacn.)[b]	358.3	1.790	11.18	
	365.5	2.022	11.15	
	383.8	2.594	11.09	
	390.6	2.964	11.24	73
(Secondary reacn.)[c]	358.3	1.557	13.94	
	365.5	1.666	13.55	
	383.8	2.653	13.96	
	390.6	2.687	13.72	73
o-Fluoro-N,N-dimethyl-	353.7[d]	0.754	15.41	
	367.6[d]	1.452	15.41	54
o-Chloro-N,N-dimethyl-	379.0[d]	1.06	...	72

[a] $k = \frac{-2.303}{[A] + [B]} \times \frac{\log (1-F)}{t}$ (l. mole^{-1} hr.$^{-1}$). [b] Exchange considered to occur at position 2; n = 1. [c] Exchange considered to occur at positions 4 and 6; n = 2. [d] For these substances the temperature control was within ±0.5°.

alcohol. After gentle warming to effect solution, 10 ml. of the scintillator solution was added from a pipet and the bottle stored in the refrigerator for 12–15 hours prior to counting.

In the case of *m*-nitro-N,N-dimethylaniline and 2,6-dimethyl-N,N-dimethylaniline, which do not form methiodides, the tritiated dialkylaniline was separated from the reaction mixture with sodium carbonate solution. The *m*-nitro compound was filtered off, washed with water and twice recrystallized from aqueous alcohol; the 2,6-dimethyl-N,N-dimethylaniline was distilled off under reduced pressure. After drying in a vacuum over sulfuric acid, the materials were counted by dissolving directly in 10 ml. of scintillator solution allowing for quenching as described above.

Equilibrium values of exchange were obtained by leaving ampoules in the bath until no change in specific activity could be detected between consecutive samples.

Typical results are shown in Table II for *p*-fluoro-N,N-dimethylaniline and kinetic data for all the compounds are summarized in Table III.

(d) **Activation Energies.**—Activation energies were computed either from large scale plots of log k against $1/T$ or by the method of least squares, whichever was most suitable. In most cases the rate constant was determined at four or more different temperatures. Typical results are shown in Table IV.

(e) **Exchange Number.**—The specific activity at exchange equilibrium was used to determine the number of exchangeable hydrogen atoms in the dialkylaniline molecule, designated in the following as the exchange number, n. This was calculated from the equation

$$n = \frac{[H]}{[R_2N]} \frac{T_{R_2N}}{T_S}$$

where

$[H]$ = molar concn. of exchangeable hydrogen in solvent
$[R_2N]$ = molar concn. of amine
T_{R_2N} = equil. activity of tritium in amine form
T_S = equil. activity of tritium in solvent

Values of equilibrium specific activity for the amines and exchange numbers are shown in Table V. With the exception of two compounds, the exchange numbers correspond very closely to values predicted from theoretical consideration of the activating influence of substituents. The low value for the *o*-chloro-N,N-dimethylaniline almost certainly arises from failure to attain true equilibrium due to the extremely slow rate of exchange.

At present we are unable to account for the measured value for 3,5-dimethyl-N,N-dimethylaniline. Although the figure of 2.2 suggests an exchange number of 2 rather than 3, the symmetrical nature of the structure in this molecule makes it difficult to accept this conclusion. It seems likely therefore that the low value is due to experimental error and this possibility is being investigated.

TABLE IV

ACTIVATION ENERGY FOR *p*-FLUORO-N,N-DIMETHYLANILINE

K^a	$1/K \times 10^3$	log k^b	Slope[c]	σ^d	r^e	$\sigma_y{}^f$
338.4	2.9551	$\bar{4}.326$				
348.0	2.8736	$\bar{4}.840$				
356.2	2.8074	$\bar{3}.232$				
363.8	2.7488	$\bar{3}.596$	−6.137	0.003	0.999	0.015

[a] Temperature in absolute degrees. [b] k second-order rate constant. [c] Least squares slope. [d] Standard deviation of slope. [e] Correlation coefficient. [f] Standard deviation in vertical direction.

TABLE V

EXCHANGE NUMBERS

Aniline	Equilibrium spec. act., c.p.s./mmole	Exchange number (n) Measured	Predicted
Dimethyl-	17500	2.7	3
Diethyl-	20500	3.1	3
Di-*n*-propyl-	17700	2.7	3
Di-*n*-butyl-	18300	2.8	3
p-Methyl-N,N-dimethyl-	11400	1.7	2
p-Methoxy-N,N-dimethyl-	10300	1.6	2
p-Fluoro-N,N-dimethyl-	11100	1.7	2
p-Chloro-N,N-dimethyl-	10800	1.7	2
p-Bromo-N,N-dimethyl-	11700	1.7	2
m-Methyl-N,N-dimethyl-	17800	2.7	3
3,5-Dimethyl-N,N-dimethyl-	14100	2.2	3
m-Ethoxy-N,N-diethyl	18200	2.8	3
m-Nitro-N,N-dimethyl	20300	3.0	3
o-Fluoro-N,N-dimethyl	10100	1.6	2
o-Chloro-N,N-dimethyl	8600	1.3	2

(f) **Determination of pK_a Values.**—The procedure used was similar to that described by Thomson.[41] The weighed amount of base was dissolved in rather less than 50 ml. of Analar ethanol in a standard flask; 50 ml. of 0.01 N aqueous hydrochloric acid was added and the volume made up to 100 ml. with ethanol.

pH values of the solution were then measured using a Pye pH meter. pK_a values for the conjugate acids were calculated from the Henderson equation

$$pK_a = p\text{H} + \log([\text{salt}]/[\text{base}])$$

Where the $p\text{H} < 4$, the equation was corrected for hydrolysis using the form

$$pK_a = p\text{H} + \log \frac{[\text{salt}] - [H^+]}{[\text{base}] + [H^+]}$$

All measurements were made at 25° and the pH meter was standardized with potassium hydrogen phthalate buffer solution at pH 4.01.

Experimental values of pK_a are shown in Table VI.

TABLE VI

BASICITIES IN 50% ETHANOL AT 25°

Aniline	w, g. used	pH obsd.	pK_a calcd.	Av. pK_a
N,N-Dimethyl-	0.1466	4.40	4.25	
	.1735	4.54	4.27	
	.2319	4.70	4.25	4.26
N,N-Di-*n*-propyl-	.2248	4.99	4.80	
	.1589	4.68	4.78	
	.1957	4.85	4.78	4.79
m-Methyl-N,N-dimethyl-	.1859	4.52	4.28	
	.1740	4.47	4.27	
	.2152	4.63	4.29	4.28
m-Nitro-N,N-dimethyl-	.1055	2.62	2.46	
	.1202	2.61	2.01	
	.2107	2.65	2.11	2.19
o-Chloro-N,N-dimethyl-	.0972	2.88	3.04	
	.1066	2.95	3.06	
	.1514	3.12	3.01	3.04
o-Fluoro-N,N-dimethyl-	.0950	2.85	2.89	
	.1065	2.95	2.96	2.93
m-Ethoxy-N,N-diethyl-	.1754	5.30	5.39	
	.1566	5.17	5.38	
	.2252	5.52	5.39	5.39

(g) **Determination of Dissociation Free Energies.**—For the equilibrium

$$RRN^+HAr \rightleftharpoons RRNAr + H^+$$

the free energy of dissociation is given by

$$-\Delta F^0 = RT \ln K_a$$

where K_a = thermodynamic dissociation constant for conjugate acid

$$\therefore \Delta F^0 = 2.303RT \times pK_a$$

Since values of pK_a for the conditions used in these experiments were not available, it was necessary to obtain the temperature coefficient of pK_a and the correction for alcohol content of the reaction medium.

Accurately weighed amine was dissolved in about 90 ml. of Analar absolute ethanol and 0.5 ml. of 0.1 N HCl was added. The solution was made up 100 ml. with Analar ethanol. pH values were measured for a series of temperatures ranging from 23° to 68°, which were kept constant to ±1.0° in a thermostatic bath. pK_a values were calculated using the Henderson equation. The Debye–Hückel correction term was small and therefore neglected.

Temperature coefficients were obtained from plots of pK_a against temperature; typical data are shown in Table VII. These were similar to those of Hall and Sprinkle,[42] who measured them in aqueous solution. This independence of temperature coefficient from solvent composition has also been confirmed by other workers.[43] In other measurements of temperature coefficient, it has been shown that for any given solvent the temperature coefficient is constant up to 80°.[44,45]

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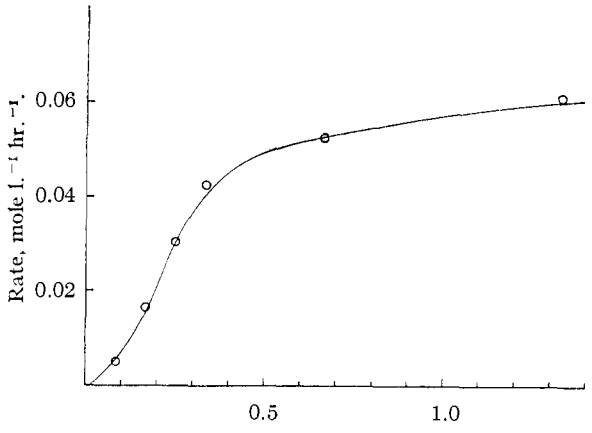


Fig. 1.—Dependence of the rate of exchange on the acid/amine ratio for N,N-dimethylaniline at 75°.

To study the effect of alcohol content on pK_a values, the required amount of distilled water was added successively to obtain 75 and 50 volume % alcohol solutions and the pH value of each solution measured (Table VIII).

TABLE VII

TEMPERATURE COEFFICIENTS OF pK_a IN 95% ALCOHOL SOLUTION

	pK_a	
Temp., °C.	N,N-Dimethylaniline	*m*-Ethoxy-N,N-diethylaniline
23	2.70	4.00
35	2.45	3.77
45	2.36	3.63
50	2.23	3.48
55	2.18	3.43
60	2.14	3.41
68	1.99	3.29
$\Delta pK_a/\Delta T$	0.0147	0.0168

TABLE VIII

EFFECT OF ALCOHOL CONTENT ON pK_a

	pK_a		
	m-Ethoxy-N,N-diethylaniline		*m*-Nitro-N,N-dimethylaniline
Alcohol, %	25°	50°	25°
50	5.10	4.88	2.31
75	4.65	4.15	1.67
95	3.90	3.62	

By plotting pK_a against alcohol content parallel straight lines were obtained for the two compounds studied. The data obtained by Hall and Sprinkle[42] for other dialkylanilines show a similar linear relationship between pK_a and alcohol content.

pK_a values from Table VI and other values from the literature were corrected for temperature and alcohol content by the coefficients obtained in this section and those given by Hall and Sprinkle.[42]

Results and Discussion

Hydrogen exchange in the dialkylaniline system seems to occur only in the presence of free acid. This had been suggested previously for deuterium exchange,[2,4] and preliminary experiments in the present work confirmed that it is also true for tritium exchange. It has been established that in the absence of acid the extent of exchange between dimethylaniline and tritiated water or ethyl alcohol is quite negligible after 24 hours at 80°. The analytical method based on measurement of radioactivity is extremely sensitive and capable of detecting very low levels of exchange so that the evidence seems to be conclusive.

The addition of even small quantities of acid to the reaction medium was sufficient to produce a measurable exchange rate. However, it was obvious that the occurrence of salt formation on addition of acid to the dialkylaniline would tend to complicate the interpretation of kinetic data obtained from this system. In order to establish conditions least likely to cause difficulty, the rate of exchange was measured for a series of acid/base ratios. These results are shown in graphical form in Fig. 1. The interpretation of this curve may be considered in relation to the equilibrium representing salt formation

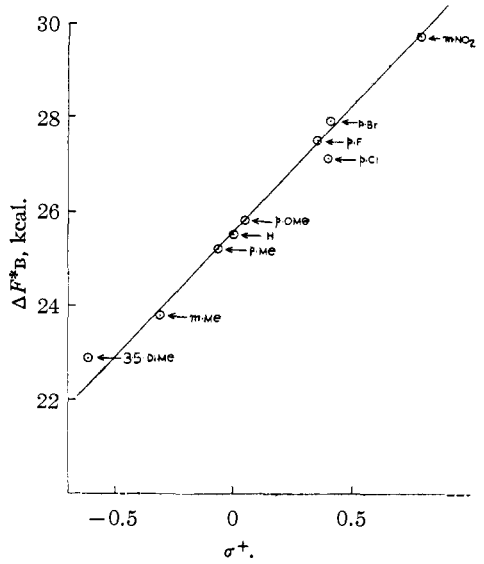


Fig. 2.—Hammett plot (ΔF^*_B value for *m*-NO_2– is the average of primary and secondary reaction).

$$H_3O^+ + RRNAr \rightleftharpoons RR\overset{+}{N}HAr + H_2O$$

Calculations from the equilibrium constant show that the region of rapidly increasing exchange rate from zero acid up to an acid/base ratio of 0.5 corresponds to steeply rising concentrations of hydroxonium ion and of conjugate acid ion ($RRNH^+Ar$) with near constant but large excess of free base molecules. In this region it seems likely therefore that the exchange rate is dependent on the hydroxonium ion concentration or, less probably, on the concentration of conjugate acid ions.

For acid/base ratios between 0.5 and 1.0 the hydroxonium ion and conjugate acid ion concentrations continue to increase but less steeply. The free base concentration falls rapidly to a very low level, reaching less than 10^{-3} M at ratio 1. The near constancy of exchange rate at acid/base ratios greater than 1 suggests that the exchange occurs through the conjugate acid ion, although later results indicate that the rate-controlling step involves the basic dialkylaniline molecule itself.

The conclusion reached from these preliminary experiments was that the kinetic studies should be conducted in solutions containing a mild excess of acid over base. Under these conditions the measured exchange rate would be largely independent of small errors in acid content and the dialkylaniline would be present almost entirely in one form, *i.e.*, the conjugate acid ion. The greater rate of exchange in this medium would also facilitate measurements of activation energy at lower temperatures.

Using these selected conditions kinetic data were obtained for a series of substituted dialkylanilines. Table IX summarizes the more important kinetic data and thermodynamic parameters.

Hammett Relationship.—The results in Table IX show that the introduction of substituents into the aromatic ring produces significant changes in the rate constants compared with that for dimethylaniline itself. It is therefore appropriate to inquire whether

TABLE IX
SUMMARY OF KINETIC AND THERMODYNAMIC DATA AT 65°

Aniline	E_A[a]	log k + 8[b]	$\Delta F^*_{BH^+}$[c]	pK_a[d]	pK_a[e] cor.	ΔF^o[f]	ΔF^*_B[g]
N,N-Dimethyl-	27.7	1.793	29.5	4.26	2.56	4.0	25.5
N,N-Diethyl-	29.8	0.845	30.9	5.85*	4.07	6.3	24.6
N,N-Di-*n*-propyl-	26.9	1.320	30.2	4.79	3.05	4.7	25.5
N,N-Di-*n*-butyl-	26.6	1.284	30.3	4.84*	3.10	4.8	25.5
p-Methyl-N,N-dimethyl-	28.7	1.543	29.9	4.77*	3.07	4.7	25.2
p-Methoxy-N,N-dimethyl-	29.5	0.756	31.1	5.16*	3.42	5.3	25.8
p-Fluoro-N,N-dimethyl-	28.1	0.761	31.1	4.01*	2.35	3.6	27.5
p-Chloro-N,N-dimethyl-	23.9	1.636	29.7	3.33*	1.71	2.6	27.1
p-Bromo-N,N-dimethyl-	23.3	1.614	29.8	2.82*	1.20	1.9	27.9
m-Methyl-N,N-dimethyl-	25.4	2.900	27.8	4.28	2.58	4.0	23.8
3,5-Dimethyl-N,N-dimethyl-	27.4	3.267	27.2	4.48[41]	2.78	4.3	22.9
m-Ethoxy-N,N-diethyl-	27.4	3.267	27.2	5.39	3.65	5.6	21.6
m-Nitro-N,N-dimethyl- (1)	22.1	1.313	30.2	2.19	0.61	0.9	29.3
(2)	26.9	0.840	30.9	2.19	0.61	0.9	30.0
o-Fluoro-N,N-dimethyl-	30.2	0.267	31.8	2.93	1.31	2.0	29.8
o-Chloro-N,N-dimethyl-	..	1.494[h]	..	3.04	1.42	2.2	..
o-Methyl-N,N-dimethyl-	Exchange negligible		90.1°	5.07[41]	3.33	5.1	..
o-Methoxy-N,N-dimethyl-	at stated temperature		88.3°	5.49*	3.73	5.8	..
2,4-Dimethyl-N,N-dimethyl-			90.7°	5.28[41]	3.54	5.5	..
2,6-Dimethyl-N,N-dimethyl-			90.5°	4.69[41]	2.97	4.6	..

[a] Kcal.; accurate to within ±0.3 kcal. [b] l. mole^{-1} sec.$^{-1}$; accurate to within ±10%. [c] Kcal.; free energy of activation for over-all reaction from conjugate acid. [d] In 50% alcohol solution at 25°. Values with * are taken from the literature reference: W. C. Davies and H. W. Addis, *J. Chem. Soc.*, 1622 (1937). Other values are also available in the literature but we have selected these since our measurements of pK_a were based on the same method as that described by these two authors. [e] Corrected to 95% alcohol solution at 65°.; [f] Kcal ; free energy change required for the dissociation of amine conjugate acid, calculated from the corrected pK_a values. [g] Kcal.; free energy of activation for free base. [h] Values given for 106°.

or not the Hammett relation applies to this reaction. Although the entropy of activation (ΔS^*) is not constant throughout the series, it can be shown that ΔS^* varies linearly with the enthalpy of activation (ΔH^*) and under these sufficient conditions application of the Hammett relationship is justified.[46-48] Hammett substituent constants[49] were separately plotted against ΔF_{BH^+}* and ΔF_B* values from Table IX representing free energies of activation for the reaction starting from the conjugate acid ion and the free base, respectively. The *meta* sigma constants σ_m were chosen for the *p*-substituted dialkylanilines and the *para* constants σ_p for those compounds containing *m*-substituents.[50] A separate test of the Hammett relation was made using the modified electrophilic substituent constants(σ^+) introduced by Brown and Okamoto.[51]

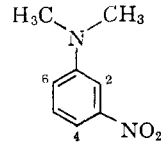
A very satisfactory Hammett plot was obtained using the modified constants with ΔF_B* and this is shown in Fig. 2. For this graph the correlation coefficient is 0.992 covering a range of σ^+-values from −0.62 to +0.79. With the normal Hammett constants (σ) the coefficient was 0.933.

When ΔF_{BH^+}* values were used, far less satisfactory plots were obtained with both σ^+ and σ constants; the correlation coefficients were 0.755 and 0.671, respectively.

From log k and σ^+-values the reaction constant ρ was calculated to be −3.54 for a mean reaction temperature of 65°.

For 3,5-dimethylaniline the value of σ^+ used in the plot is just twice the value of a single *m*-methyl group. The small divergence of the point from the straight line may indicate that simple additivity is not wholly justified. From Fig. 2 the σ_p^+ constant for ethoxy is −0.72; this does not seem to have been recorded elsewhere.

For *m*-nitro-N,N-dimethylaniline the mean of the two ΔF_B* values was used in the Hammett plot. The two values arise from the complex exchange rate curve obtained at each temperature for this compound. Each curve was analyzed into two component rates, which it is thought represent the exchange of non-equivalent hydrogen atoms in the 2-, 4- and 6-positions.



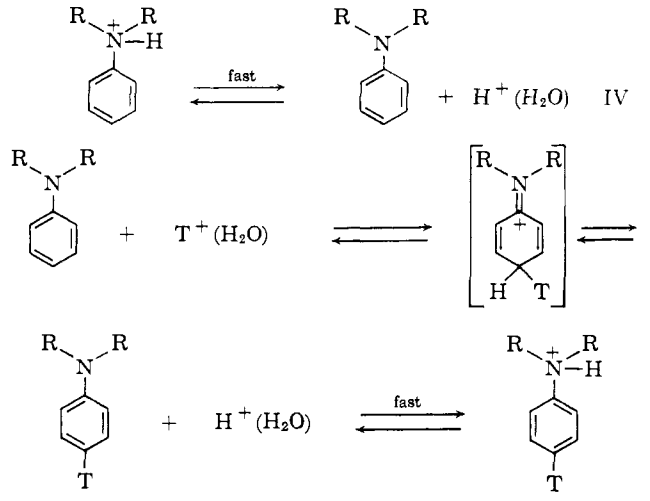
It is not obvious which positions exchange most rapidly, but by analogy with other substitution reactions in similar aromatic systems[52,53] it seems probable that the 2-position undergoes the more rapid exchange and the 4- and 6-positions exchange at an equivalent but slower rate.

Reaction Mechanism.—The data presented here are too limited to allow any firm conclusions to be drawn about reaction mechanism and a further communication dealing with mechanism will follow shortly. However, it is useful to consider briefly how the present results influence the choice of a possible mechanism.

For the range of compounds investigated, the rate constants vary between extremes by a factor of 1000 (*o*-fluoro and *m*-ethoxy) and Arrhenius activation energies lie between 30.2 and 22.1 kcal. (*o*-fluoro and *m*-nitro). Inspection of the actual values of E_A at first seems to preclude an electrophilic substitution mechanism. Thus for the electron-releasing 3,5-dimethyl-N,N-dimethylaniline, E_A is of the same order as the corresponding unsubstituted dimethylaniline, while for the *m*-nitro, *p*-chloro and *p*-bromo compounds E_A is significantly lower. These apparent anomalies lead to the failure of the Hammett relationship when log k or ΔF_{BH^+}* is plotted against substituent constant.

(46) J. E. Leffler, *J. Org. Chem.*, **20**, 1202 (1955).
(47) J. E. Leffler, *J. Chem. Phys.*, **23**, 2199 (1955).
(48) A. Fischer and J. Vaughan, *ibid.*, **27**, 976 (1957).
(49) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chap. VII.
(50) H. H. Jaffé, *Chem. Revs.*, **53**, 191 (1953).
(51) H. C. Brown and Y. Okamoto, *J. Am. Chem. Soc.*, **80**, 4979 (1958).
(52) A. F. Holleman, *Rec. trav. chim.*, **22**, 263 (1903).
(53) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 268.

However, these difficulties can be removed by relating the data to the conditions in which the reaction was studied. As mentioned previously, in all experiments excess mineral acid was used so that the dialkylanilines were present almost entirely in the conjugate acid form ($RR\overset{+}{N}HAr$). Although it is just possible that this ionic species may undergo attack by hydroxonium ion to give *m*-substitution, another more plausible interpretation is available which recognizes the dynamic equilibrium existing in solution between conjugate acid ion and basic molecules of the dialkylaniline. If it is assumed that it is the latter which undergoes the exchange reaction, the reaction sequence may then be represented as



In these circumstances the experimental kinetic data represent both steps of the over-all reaction so that the activation energy term E_A includes the enthalpy of dissociation and the activation enthalpy of exchange. Similarly, the free energy of activation $\Delta F_{BH^+}{}^*$ arises from the summation of the free energy of dissociation ΔF^0 and the activation free energy of exchange $\Delta F_B{}^*$. Calculation from $\Delta F_{BH^+}{}^*$ and pK_a values leads to a series of $\Delta F_B{}^*$ values which give the satisfactory Hammett plot (Fig. 2).

Steric Effects of *o*-Substituents.—An attempt was made to obtain quantitative data about the degree of steric inhibition of resonance caused by *o*-substituents in the dialkylaniline system. Table IX shows that most of the *o*-substituted compounds failed to undergo exchange at the highest temperatures attainable and positive results were obtained only for the *o*-fluoro and *o*-chloro compounds, but in the latter case exchange equilibrium was only reached after 60 days at 106°. In view of the paucity of useful data from these experiments it seemed that an investigation of the exchange in dialkylanilines containing bulky alkyl groups would be justified. Preliminary trials indicated that for di-*n*-propylaniline exchange occurred at a measurable rate, even though molecular models suggested a fair degree of steric interference between the propyl groups and the *o*-hydrogen atoms on the aromatic ring.

The results obtained do not provide direct evidence of steric inhibition of resonance and it may well be that the only unambiguous evidence is that from the last four compounds in Table IX, which do not undergo measurable exchange. This almost certainly is due to fairly complete steric inhibition of resonance through the C–N bond by the large *ortho* groups.

In the region between complete inhibition and complete freedom of rotation it is much more difficult to demonstrate partial effects. The *o*-fluoro compound for which evidence of partial inhibition of resonance has been claimed[5] is typical of the ambiguity.

The $\Delta F_B{}^*$ values for N,N-dimethylaniline, *p*-fluorodimethylaniline and *o*-fluorodimethylaniline are, respectively, 25.5, 27.5 and 29.8 kcal. The increase of 2 kcal. for the *p*-fluoro compound probably is due to the strong inductive electron attraction exerted by the fluorine atom. The further increase of 2.4 kcal. for *o*-fluoro can be accounted for either by partial steric inhibition of resonance, or by the enhanced inductive effect when fluorine is present in the *o*-position. The similarities of entropy of activation, $\Delta S_B{}^*$ (Table X), for the two compounds rather supports the view that any steric effect must be small.

TABLE X

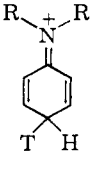
SOME THERMODYNAMIC PARAMETERS

Aniline	$-\Delta S_{BH^+}{}^*$	$\Delta F_B{}^*$	$-\Delta S_B{}^*$	$\Delta H_B{}^*$	$\log k + 8$
Dimethyl-	7.4	25.5	18.3	19.3	1.793
Diethyl-	5.3	24.6	13.0	20.2	0.845
Di-*n*-propyl-	11.8	25.5	21.9	18.1	1.320
Di-*n*-butyl-	13.0	25.5	22.8	17.8	1.284
o-Fluorodimethyl-	6.8	29.8	21.0	22.7	0.267
p-Fluorodimethyl-	10.9	27.5	22.8	19.8	0.761

For the *o*-chloro compound the rate constant at only one temperature (106°) is available at present, so that conclusions must be provisional. Assuming an activation energy of 30 kcal. (which is probably too low) the $k_{65°}$ constant is about one-quarter of the rate for *o*-fluoro, giving an activation free energy ($\Delta F_{BH^+}{}^*$) of 33–34 kcal. The inductive electron attraction by an *o*-chlorine atom is not likely to exceed that for *o*-fluorine (pK_a values are similar), so that the high activation free energy can with some confidence be attributed to partial steric inhibition of resonance.

The results for the N,N-dialkyl series are inconclusive and the differences in rate constants, although significant, are not large. For dimethyl, di-*n*-propyl and di-*n*-butyl the rate seems to be strongly controlled by the entropy of activation. This is illustrated by reference to Table X, where it is found that the rate constants follow the direction of entropy changes ($\Delta S_B{}^*$). The low measured rate constant for N,N-diethylaniline is undoubtedly caused by its high basicity giving rise to the high activation energy (E_A) for the over-all reaction, but when this has been corrected for, once again the importance of activation entropy is revealed.

The prominence of the entropy term may be accounted for by steric factors which influence both the reactant and the transition state complex



For example, inductive electron release from the dialkyl groups may be expected to stabilize the transition complex, but in the di-*n*-propyl and di-*n*-butyl compounds the steric strain caused by these bulky alkyl groups will tend to counteract this stabilization.

Further, it seems probable that the solvation of the reactants and the transition state will be centered on the nitrogen atom, and if this is so, the same steric and inductive effects of the N,N-dialkyl groups may combine to account for the anomalous position of N,N-

diethylaniline. Certainly molecular models indicate that if free rotation is preserved about all bonds in di-*n*-propyl and di-*n*-butyl groups considerable steric crowding occurs around the nitrogen atom, thus making solvation more difficult.

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[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND BIOLOGY, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE 18, MD.]

The Structure and Synthesis of Firefly Luciferin[1]

BY EMIL H. WHITE, FRANK MCCAPRA AND GEORGE F. FIELD

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Luciferin from the common American firefly, *Photinus pyralis*, was shown to be D-2-(6-hydroxy-2-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid (XXII). The structure was confirmed by a total synthesis as outlined in the text. L-Luciferin, DL-luciferin, dehydroluciferin and various related benzothiazoles were also synthesized.

A surprisingly large number of plant and animal species are bioluminescent.[2] The bioluminescence proper has been studied in only a few cases, however, despite the fact that interest in this subject dates back to the beginning of the scientific era with the work of Francis Bacon on luminous fungi and Robert Boyle on luminous bacteria.[3] From the systems that have been studied (these include the firefly *Photinus pyralis*, the crustacean *Cypridina hilgendorfii* and the bacterium *Photobacterium fischerii* as perhaps the best known examples),[2,4] the generalization can be drawn that light emission in bioluminescence is a result of the reaction of oxygen with an oxidizable substrate (a luciferin) catalyzed by an enzyme (a luciferase). In this paper we report the proof of structure and synthesis of luciferin from the common American firefly *Photinus pyralis*.[5] Firefly luciferin is at the present time the only luciferin of known structure.[6]

Luciferin.—Firefly luciferin[7] was first isolated in 1957 and a molecular formula of $C_{13}H_{12}N_2O_3S_2$ was assigned to it at that time.[4a] During the present work, since luciferin was difficult to purify and since very small quantities were available for investigation (we estimate that a total of 30 mg. was used for the structure determination), elementary analyses were not obtained for luciferin or for its degradation products with the one exception noted below. A molecular formula of $C_{11}H_8N_2O_3S_2$ was nevertheless assigned to luciferin during the present work, largely on the basis of indirect evidence from the degradation and from the analysis of a derivative of dehydroluciferin; a total synthesis of luciferin later showed that this formula was correct.

Luciferin is a pale-yellow, microcrystalline solid, m.p. 190° dec. It is difficult to recrystallize, it cannot be sublimed without decomposition, and it is unstable to acids, oxygen and light. Luciferin is stable in basic solutions free of oxygen, but in similar solutions containing oxygen it is rapidly oxidized to a derivative, dehydroluciferin. Spot tests on luciferin indicated the presence of a group that coupled readily with diazonium ions, and the absence of the thiol, thioketone, disulfide and basic nitrogen groups.

The ultraviolet spectrum of natural luciferin in water showed maxima at 265 mμ (broad, log ε 3.90) and 327 mμ (log ε 4.30). These bands were displaced in alkaline solutions to 283 mμ (log ε 3.88) and 383 mμ (log ε 4.27) with a pK_a for the change of 8.4. Similar values for the pK_a were obtained from shifts of electrophoretic mobility, fluorescence intensities and fluorescence excitation with *p*H.[4a,8] These results and the spot test data suggested that a phenolic group was present in luciferin.

Luciferin is highly fluorescent in solution, a fact in accord with its rigid and highly conjugated structure. In neutral solutions, luciferin fluoresces at 535 mμ (excitation at 327 mμ); in basic solutions it also fluoresces at 535 mμ (excitation at 385 mμ), but the emission intensity is about four times as great. Apparently the phenolate ion is the emitter in both cases, and the phenol which is excited in neutral or acid solutions loses a proton before it fluoresces.[9]

The infrared spectrum of luciferin showed, in particular, a broad band in the OH stretching region and a single band in the carbonyl region. A few samples of natural luciferin showed two carbonyl bands, but a later comparison with synthetic material indicated that the second band probably was due to some racemic luciferin in the samples. The low observed rotation ($[\alpha]_D$ −0.6°) and the abnormally low activity in the enzymatic assay of "highly purified" natural luciferin also suggested that our isolation procedure had led to partial racemization. The carbonyl band was assigned to a carboxyl group[4a] since luciferin reacted instantly with diazomethane to give an ester, and with ammonia to give a salt with a band in the infrared at 6.3 μ, a position characteristic of carboxylate ions. The three oxygen atoms of luciferin were therefore accounted for by the phenol and carboxylic acid groups.

Dehydroluciferin[10] was prepared by the oxidation of luciferin in basic solutions with either potassium ferricyanide or oxygen. Dehydroluciferin has also been isolated from firefly tails[4a] and it can be seen on paper chromatograms of the whole light organs of certain

(1) Presented in part before the Steroids and Other Natural Products Section of the Gordon Research Conferences, New Hampton School, New Hampton, N. H., August, 1961. A preliminary communication on this subject appeared in *J. Am. Chem. Soc.*, **83**, 2402 (1961).

(2) E. N. Harvey, "Bioluminescence," Academic Press, Inc., New York, N. Y., 1952.

(3) E. N. Harvey, "A History of Luminescence," The American Philosophical Society, Philadelphia, Pa., 1957.

(4) (a) B. Bitler and W. D. McElroy, *Arch. Biochem. and Biophys.*, **72**, 358 (1957); (b) W. D. McElroy and H. H. Seliger, "Light and Life," The Johns Hopkins Press, Baltimore, Md., 1961, p. 219; (c) Y. Haneda, *et al.*, *J. Cellular Comp. Physiol.*, **57**, 55 (1961); (d) W. D. McElroy, J. W. Hastings, V. Sonnenfeld and J. Coulombre, *J. Bacteriol.*, **67**, 402 (1954).

(5) The different species of fireflies apparently contain the same luciferin (enzymatic tests; see ref. 2). Our paper chromatographic results with single fireflies (males) supports this conclusion, at least insofar as the local species are concerned. The local species of fireflies are characterized in "The Fireflies of Delaware" by F. A. Mcdermott (Society of Natural History of Delaware, Wilmington, Del., 1958).

(6) A structure has been proposed for Cypridina luciferin by Y. Hirata, O. Shimomura and S. Eguchi (*Tetrahedron Letters*, No. **5**, 4 (1959)); however, it is incorrect in part (private communication, O. Shimomura). Cypridina luciferin is an indole derivative and its structure is apparently quite different from that of firefly luciferin.

(7) Referred to as luciferin in the remainder of the paper.

(8) B. L. Strehler, "The Luminescence of Biological Systems," American Association for the Advancement of Science, Washington, D. C., 1955, p. 199.

(9) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).

(10) Previously called Oxyluciferin (ref. 4a).