

TABLE I
TRACER STUDIES ON THE CATALYTIC HYDROLYSIS OF UREA
BY UREASE

Expt.	Reaction mixture (in H ₂ O ¹⁸ -enriched 0.5 M citrate buffer at pH 5.5)	Temp., °C.	Reaction time	Atom % of O ¹⁸ in the liberated CO ₂
1	Urease + urea + buffer ^a	0	0.5 min.	0.842
2	Urease + urea + buffer ^a	0	0.5 min.	0.873
3	Urease + urea + buffer + octanol ^b	0	0.5 min.	0.873
4	Urease + urea + buffer ^a	~20	13.5 hr.	1.40
5	Urease + urea + buffer ^a	~20	8 hr.	1.52
6	Urease + urea + buffer ^a	0, 100, 20°	12.5 hr.	1.47
7	Urease + urea + buffer + octanol ^b	0, 100, 20°	6 hr.	1.50
8	Urea + buffer + NaHCO ₃ ^d	0	0.5 min.	0.224
9	Urea + buffer + NaHCO ₃ ^d	0	0.5 min.	0.219

^a The concns. are: urea, 0.25 M; jack bean urease, 0.08 g./ml. ^b A droplet of octanol was added to the mixture to prevent foaming caused by the liberation of CO₂. ^c In these experiments the reactants were mixed for 1/2 min. at 0°, heated at 100° for 4.5 min. and then left at room temperature (~20°) for several hr. to reach equilibrium. ^d 25 mg. of dry NaHCO₃ powder was used to react with 0.6 ml. of 0.25 M urea in H₂O¹⁸-enriched citrate buffer soln.

enrichment ratio is equal to $(0.86 - 0.22)/(1.47 - 0.22) = 0.51$, *i.e.*, approximately equal to 1/2.

Therefore it may be concluded that of the three mechanisms of urease action discussed in the literature, only the *carbamic acid mechanism* is consistent with the experimental results for slightly acid solutions obtained in the present work.

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Acid-Base Reactions in Non-dissociating Solvents *n*-Butylamine and Acetic Acid in Carbon Tetra- chloride and Chloroform

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Ion-pair structures for model systems containing a tertiary¹ and a secondary² amine and acetic acid have been reported recently from this Laboratory. The observation of the *n*-butylamine-acetic acid system completes this series.

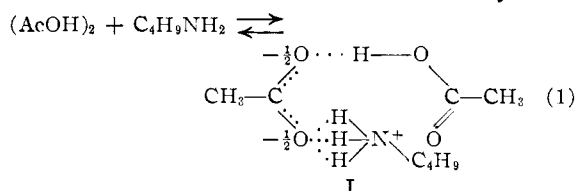
Experimental

The instrumentation,² and the purification of the solvents and acetic acid,³ and the preparation of the solutions² have been described previously. Eastman Kodak *n*-butylamine was distilled after drying over KOH and the constant boiling center cut used. Spectra were obtained in appropriate cells for acetic acid concentrations of 0.3, 0.1, 0.02 and 0.0008 M with the addition of various quantities of *n*-butylamine.

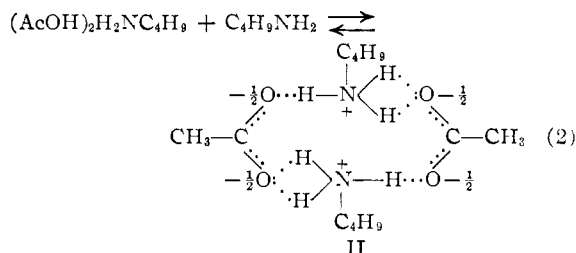
The basis for the interpretation of the spectra is the same as in the diethylamine-acetic acid system previously discussed.² With the exception of the 5.1 μ amine salt band which will be discussed later, the behavior of the bands in the *n*-butylamine-

acetic acid system parallels that of the bands in the diethylamine-acetic acid system. Thus analogous species are postulated for the present system.

In concentrated CCl₄ and CHCl₃ solutions an intermediate, (AcOH)₂H₂NC₄H₉, characterized by both 5.85 and 6.5 μ bands, is formed initially

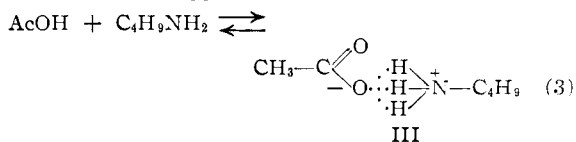


Structure I is suggested by analogy with the diethylamine-acetic acid system. Further addition of *n*-butylamine leads to elimination of the 5.85 μ band and replacement of the 6.5 μ band by one at 6.4 μ in both CCl₄ and CHCl₃. The formation of the salt dimer, (AcOH₃NC₄H₉)₂, with some type of hydrogen bonded bridge, depicted as structure II, is thus postulated

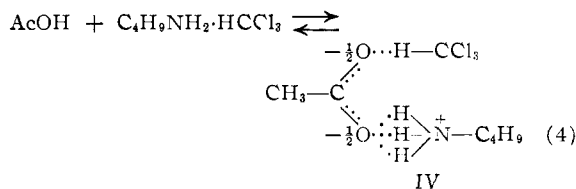


For the *n*-butylamine system the broad 5.1 μ band, probably an H₃N⁺ deformation vibration, appears characteristic only of the intermediate, I, and is replaced by a similar band at 4.6 μ in the final product, II. This behavior contrasts the diethylamine case where the 5.1 μ amine salt band was found for both the intermediate and the final product. This shift may be interpreted as evidence for a different arrangement of the hydrogen bonding of the *n*-butylammonium ion in species II as compared with that of species I.

In the most dilute solutions the spectra and the equilibrium constants indicate direct formation of a salt monomer, AcOH₃NC₄H₉. In CCl₄ a carbonyl band at 5.9 μ, but no free N-H stretching band, is observed for the salt monomer, hence the formation of III is suggested



In CHCl₃ solution no carbonyl band is observed for the salt, therefore structure IV, which would be expected to show only a carboxylate band,^{1,2} is postulated for the salt monomer.



(1) G. M. Barrow and E. A. Yerger, *THIS JOURNAL*, **76**, 5211 (1954).

(2) E. A. Yerger and G. M. Barrow, *ibid.*, **77**, 4474 (1955).

(3) G. M. Barrow and E. A. Yerger, *ibid.*, **76**, 5248 (1954).

The equilibrium constants for direct salt formation, reactions 3 and 4, were calculated from the intensity of the acetic acid monomer carbonyl band and the acetic acid dimerization constant,³ Tables I and II. As before, reaction 1 appears almost quantitative and its equilibrium constant, therefore, could not be satisfactorily calculated. Reaction 2 is also nearly quantitative but constants of 150 ± 50 l. mole⁻¹ were approximated for the reaction in both solvents. No attempt was made to calculate a dimerization constant since the solvent and the excess amine interfere with the bands necessary for the calculation.

A comparison of the equilibrium constants for direct salt formation is shown in Table III. Although the base strength of the amines is expected to decrease for the series studied, the constants in CCl₄ indicate an apparent increase. This enhancement of apparent base strength probably reflects the increase in the ability of the alkyl ammonium ion to solvate the acetate ion with the formation of a diprotonic bond in diethylammonium acetate and a triprotonic bond in *n*-butylammonium acetate. In CHCl₃ this effect is superimposed on the ability of the CHCl₃ to solvate the ion-pairs and the amines. The latter effect has been determined quantitatively as the heat of solvation of the

amine in CHCl₃,⁴ Table III. Thus ion-pair structures and solvation play a prominent role in determining the apparent base strength of amines in non-dissociating solvents.

Examination of the monomer ion-pair structures presented for systems containing acetic acid and primary, secondary and tertiary amines indicates that hydrogen bonding occurs between the alkylammonium ion and one oxygen atom of the acetate ion. For salts of primary and secondary amines, where more than one acidic hydrogen atom is available, multiple hydrogen bonding to a single oxygen atom occurs. In the more concentrated solutions of the primary and secondary amine salts, the multiple hydrogen bonding of the ions appears to lead to the formation of bridged dimers.

(4) M. Tamres, S. Searles, E. M. Leighly and D. W. Mohrman, *THIS JOURNAL*, **76**, 3983 (1954).

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Polarographic Studies on the Bipyridines

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No polarographic studies appear to have been previously reported on the six isomeric bipyridines.² Pyridinium ion produces a catalytic hydrogen wave at the dropping mercury electrode; in phosphate buffers (*pH* 6.1 or 7.4) pyridine is reduced ($E_{1/2} = -1.69$ vs. S.C.E.) apparently by a six-electron process.³ Smith⁴ reduced bipyridines with hydrogen and platinum oxide in acid medium and also with tin and hydrochloric acid; increasing difficulty of reaction was reported as 4,4', 2,4', 2,2', 3,3'. The reduction of 4,4'- and quaternary bases derived therefrom has been of interest in developing oxidation-reduction indicators.^{5,7}

Experimental

Materials.—2,2'- was purchased from Eastman Kodak Co. and 4,4'- from the British Drug House. 4,4', 2,2'- and 2,4'- were prepared by the sodium-pyridine reaction⁸ and pyrolysis of pyridine⁹ yielded 2,3'- and 2,4'-. *p*-Phenanthroline (prepared by Skraup synthesis) was oxidized¹⁰ to make 3,3'. Melting points of the bipyridines and their picrates plus λ_{max} and ϵ_{max} ultraviolet values agreed well with literature values.^{4,5,11} Analytical reagent grade chemicals were used in preparing buffers which were all 0.10 *M* in

(1) Titanium Zirconium Co., Inc., Flemington, N. J.

(2) 2,2'-Bipyridine, 3,3'-bipyridine, 4,4'-bipyridine, 2,3'-bipyridine, 2,4'-bipyridine, 3,4'-bipyridine. No work is reported here on 3,4'- as we did not have a sample. For brevity we omit bipyridine in all subsequent references in this paper.

(3) P. C. Tompkins and C. L. A. Schmidt, *J. Biol. Chem.*, **143**, 643 (1942); M. Shikata and I. Tachi, *Mem. Coll. Agr. Kyoto Imp. Univ.*, **4**, 19 (1927); E. Knobloch, *Collection Czech. Chem. Commun.*, **12**, 407 (1947); M. K. Shchennikova and I. A. Korshunov, *J. Phys. Chem.*, (U.S.S.R.), **22**, 503 (1949).

(4) C. A. Smith, *THIS JOURNAL*, **50**, 1936 (1928); **53**, 277 (1931).

(5) Smith called this 3,4'- but Krumholz [*THIS JOURNAL*, **73**, 4449 (1951)] has shown it is 2,4'-.

(6) L. Michaelis and E. Hill, *J. Gen. Physiol.*, **16**, 859 (1933); *THIS JOURNAL*, **55**, 1481 (1933).

(7) H. Kuhn, *Helv. Chim. Acta*, **32**, 2247 (1949).

(8) C. A. Smith, *THIS JOURNAL*, **46**, 414 (1924).

(9) C. Krumholz, *Selecta Chim.*, No. 8, 3 (1949); *cf. C. A.*, **44**, 3992; (1950).

(10) C. A. Smith, *THIS JOURNAL*, **52**, 397 (1930).

(11) C. Krumholz, *ibid.*, **73**, 3487 (1951).

TABLE I

THE EQUILIBRIUM CONSTANT FOR REACTION 3 IN CCl₄
(ACETIC ACID 0.0008 *M*)

<i>M</i> C ₄ H ₉ NH ₂	log (<i>I</i> ₀ / <i>I</i>) 5.66 μ	$C \times 10^4$				<i>K</i>
		HOAc	(HOAc) ₂	AcOH ₂ ⁺ NC ₄ H ₉	C ₄ H ₉ ⁺ NH ₂	
0.0000	0.359	(6.37)	(0.81)
.0008	.288	5.11	.52	1.85	6.15	590
.0016	.237	4.20	.35	3.10	12.90	570
.0020	.212	3.77	.28	3.67	16.33	600
.0032	.176	3.12	.19	4.50	27.50	530
.0040	.128	2.28	.10	5.52	34.48	700
.0048	.130	2.31	.11	5.47	42.53	560
.0060	.098	1.74	.06	6.14	53.86	660
.0080	.071	1.26	.03	6.68	73.32	720
						Av. 600

TABLE II

THE EQUILIBRIUM CONSTANT FOR REACTION 4 IN CHCl₃
(ACETIC ACID 0.0008 *M*)

<i>M</i> C ₄ H ₉ NH ₂	log (<i>I</i> ₀ / <i>I</i>) 5.71 μ	$C \times 10^4$				<i>K</i>
		HOAc	(HOAc) ₂	AcOH ₂ ⁺ NC ₄ H ₉	C ₄ H ₉ ⁺ NH ₂	
0.0000	0.220	(3.40)	(2.30)
.0008	.156	2.44	1.19	3.18	4.82	2650
.0016	.112	1.73	0.60	5.07	10.93	2680
.0024	.078	1.21	.29	6.21	17.79	2880
.0032	.059	0.91	.17	6.75	25.25	2940
.0064	.029	0.45	.04	7.47	56.53	2930
						Av. 2800

TABLE III

EQUILIBRIUM CONSTANTS FOR THE REACTION OF AMINES
WITH 0.001 *M* ACETIC ACID IN CCl₄ AND CHCl₃ SOLUTIONS

Amine	<i>K</i> (CCl ₄)	<i>K</i> (CHCl ₃)	ΔH^\ddagger (cal. mole ⁻¹)
Triethylamine ¹	800	3000	870
Diethylamine ²	2800	3000	886
<i>n</i> -Butylamine	2800	600	714
Pyridine ³	200	60	484

¹ To be published.