

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, URBANA, ILL.]

## Benzimidazole Syntheses by Oxidative Cyclization with Peroxytrifluoroacetic Acid

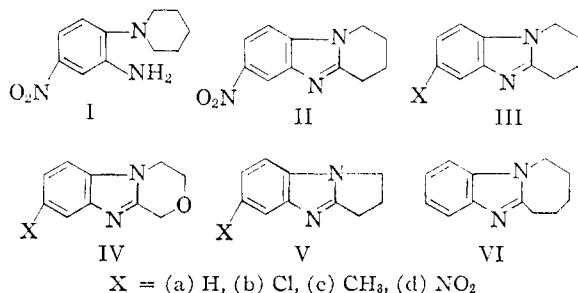
BY M. D. NAIR AND ROGER ADAMS

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A new method for the synthesis of piperidino-, pyrrolidino-, morpholino- and hexahydroazepino-benzimidazoles is described. The reaction involves oxidative cyclization of the appropriate aromatic amines with peroxytrifluoroacetic acid.

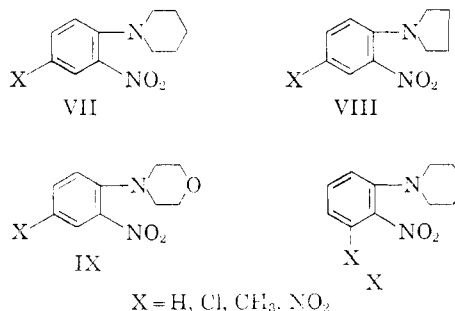
Peroxytrifluoroacetic acid has been shown to be a useful reagent for the oxidation of aromatic amines to nitro compounds,<sup>1</sup> olefins to epoxides,<sup>2</sup> ketones to esters,<sup>3</sup> and N-nitroso compounds to N-nitro compounds.<sup>4</sup> This reagent represents a distinct improvement over Caro acid or peracetic acid for similar oxidations; for example, oxidation of aniline with Caro acid is reported to give 11% of nitrobenzene in contrast to yields up to 89% realized by use of peroxytrifluoroacetic acid.

Oxidative cyclization of N-(2-amino-4-nitrophenyl)-piperidine (I) to the piperidino-nitrobenzimidazole (II) has been reported using Caro acid.<sup>5</sup> A similar oxidation of N-(2-aminophenyl)-piperidine was unsuccessful. The ring system II has been synthesized by several other methods. Saunders<sup>6</sup> prepared piperidino- morpholino- and hexahydroazepino-benzimidazoles by diazotization of the appropriate aromatic amine, such as I, conversion to the azido compound, and then pyrolysis in nitrobenzene. Piperidino<sup>7</sup> and pyrrolidino<sup>8</sup> benzimidazoles result from condensation of *o*-phenylenediamine with  $\delta$ -valerolactone and  $\gamma$ -butyrolactone, respectively. Huisgen and Rist<sup>9</sup> obtained a 16% yield of piperidinobenzimidazole by the reaction of N-lithium piperidine and nitrobenzene; it was postulated that the formation of the tricyclic product occurs through an intermediate nitroso compound.



This communication describes the successful use of peroxytrifluoroacetic acid for the facile conversion of systems such as I to the tricyclic product II. Reaction of suitably substituted *o*-nitrochlorobenzenes with the various heterocycles, piperidine, pyrrolidine, morpholine and hexamethylenimine, gives the corresponding condensation products VII-X in good yields. These nitro compounds were

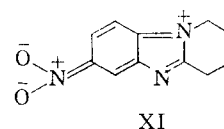
reduced to the aromatic amines catalytically or chemically. When tin and concentrated hydrochloric acid was employed, the aromatic amine was always accompanied by traces of cyclized material.



Oxidation of the aromatic amines was carried out with peroxytrifluoroacetic acid under very mild conditions. The yield of cyclic product was found to depend on the type of substituent present in the aromatic ring. The oxidation of the aniline from compound VII in which X = NH<sub>2</sub> was unsuccessful in that only tarry material resulted; but when X = H, Cl or CH<sub>3</sub>, the yields were good and when X = NO<sub>2</sub> the yields were excellent. This result is in agreement with the earlier observation<sup>1</sup> that aromatic amines substituted with electron-donating groups are not readily oxidized to nitro compounds, whereas those substituted with electron-withdrawing groups are oxidized with great ease.

Since peroxytrifluoroacetic acid prepared *in situ* using trifluoroacetic acid (or anhydride) and 30% hydrogen peroxide gave satisfactory results, experiments with 90% hydrogen peroxide were not attempted. No advantage in using methylene chloride or any other solvent for the reaction was noted.

The cyclic products formed salts with acids and the free bases could be recovered by treating with alkali, thus indicating that no permanent tautomeric shift of the double bond had occurred. The benzimidazoles in which X = H, Cl or CH<sub>3</sub> had absorption patterns in the ultraviolet similar to that of unsubstituted benzimidazole. The compounds in which X = NO<sub>2</sub> exhibited only a single maximum and the extinction was much higher (Table IV). This difference may be explained by assuming contribution of resonance forms, such as XI.



- (1) W. D. Emmons, *J. Am. Chem. Soc.*, **76**, 3470 (1954).
- (2) W. D. Emmons, A. S. Pagano and J. P. Freeman, *ibid.*, **76**, 3472 (1954).
- (3) W. D. Emmons and G. B. Lucas, *ibid.*, **77**, 2287 (1955).
- (4) W. D. Emmons, *ibid.*, **76**, 3468 (1954).
- (5) L. Spiegel and H. Kaufmann, *Ber.*, **41**, 682 (1908).
- (6) K. H. Saunders, *J. Chem. Soc.*, 3275 (1955).
- (7) W. L. Mosby, *J. Org. Chem.*, **24**, 419 (1959).
- (8) W. Reppe, *et al.*, *Ann.*, **596**, 209 (1955).
- (9) R. Huisgen and H. Rist, *ibid.*, **594**, 159 (1955).

TABLE I

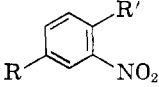
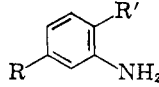
											
R	R'	Method	M.p., °C., or b.p. (mm.)	Yield, %	Solvent for recrystn.	Carbon, %		Hydrogen, %		Nitrogen, %	
						Calcd.	Found	Calcd.	Found	Calcd.	Found
H	NC <sub>4</sub> H <sub>9</sub>	A	105-107 (0.1) $n_D^{25}$ 1.6275	65		62.4	62.48	6.2	6.25	14.5	13.93
Cl	NC <sub>4</sub> H <sub>9</sub>	A	70-71	85	Aq. EtOH	52.99	53.08	4.89	5.21		
CH <sub>3</sub>	NC <sub>6</sub> H <sub>10</sub>	A	109-110 (0.1) $n_D^{25}$ 1.5744	89		65.43	65.55	7.32	7.25		
	NC <sub>4</sub> H <sub>9</sub> O	A	119-120 (0.2) $n_D^{25}$ 1.5744	68		59.4	59.22	6.32	6.11	12.6	12.38
	NC <sub>4</sub> H <sub>9</sub>	A	58-59	85	EtOH	64.05	63.78	6.84	6.75		
NO <sub>2</sub>	NC <sub>4</sub> H <sub>9</sub>	B	102	95	Aq. EtOH	50.63	50.65	4.67	4.72		

TABLE II

											
R	R'	Method	M.p., °C., or b.p. (mm.)	Yield, %	Carbon, %		Hydrogen, %		Nitrogen, %		
					Calcd.	Found	Calcd.	Found	Calcd.	Found	
H	NC <sub>4</sub> H <sub>9</sub>	A	73-75 (0.07) $n_D^{25}$ 1.4542 Unstable	82.5	74.03	73.44	8.7	8.74			
Cl	NC <sub>4</sub> H <sub>9</sub>	C	108-109 (0.2) $n_D^{25}$ 1.5982	87	61.06	61.20	6.66	6.43	14.20	14.08	
CH <sub>3</sub>	NC <sub>6</sub> H <sub>10</sub>	A	108-109 (0.4)	85	75.73	75.63	9.53	9.56	14.73	14.93	
	NC <sub>4</sub> H <sub>9</sub> O	A	129-130	83.5 <sup>a</sup>	68.70	68.71	8.38	8.46	14.57	14.62	
	NC <sub>4</sub> H <sub>9</sub>	B	89-90 (0.4)	89	74.95	75.04	9.15	9.10			
NO <sub>2</sub>	NC <sub>4</sub> H <sub>9</sub>	D	79-80	52 <sup>a</sup>	59.75	58.05	6.32	6.23	20.28	20.08	

<sup>a</sup> Recrystallized from benzene-petroleum ether (b.p. 30-50°).

TABLE III

Compound	Yield, <sup>a</sup> %	M.p., °C.	Solvent for recrystn.	Carbon, %		Hydrogen, %		Nitrogen, %		Ref.
				Calcd.	Found	Calcd.	Found	Calcd.	Found	
IIIa	58	99-100	C <sub>6</sub> H <sub>12</sub>							6, 7, 9
IVa	73	129-130	C <sub>6</sub> H <sub>12</sub>	68.95	69.01	5.79	5.81	16.08	16.14	6
Va	81	114-115	C <sub>6</sub> H <sub>6</sub> -petr. eth. <sup>b</sup> (b.p. 39-50°)	75.92	75.79	6.36	6.26			8
IIIb	65.7	152	C <sub>6</sub> H <sub>12</sub>	63.9	63.96	5.3	5.43			6
IVb	62	196	EtOAc	57.6	57.49	4.3	4.48			6
Vb	75	133-134	C <sub>6</sub> H <sub>6</sub> -petr. eth. <sup>b</sup> (b.p. 39-50°)	62.34	62.49	4.70	4.58			
IIIc	60	126	C <sub>6</sub> H <sub>12</sub>	77.37	77.27	7.57	7.53			
IVc	61	170-171	C <sub>6</sub> H <sub>6</sub> -C <sub>6</sub> H <sub>12</sub>	70.18	70.26	6.42	6.39	14.88	14.78	
Vc	86	144	CH <sub>3</sub> COC <sub>2</sub> H <sub>5</sub> , subl.	76.72	77.00	7.02	6.96	16.24	15.98	
IIId	95	219-220	CH <sub>3</sub> COC <sub>2</sub> H <sub>5</sub>	60.81	60.79	5.1	4.96	19.34	19.33	5, 6
IVd	76	214-215	CH <sub>3</sub> COC <sub>2</sub> H <sub>5</sub>	54.78	54.68	4.13	4.28	19.1	18.74	6
Vd	72	209-210	CH <sub>3</sub> COC <sub>2</sub> H <sub>5</sub>	59.10	59.56	4.46	4.68	20.68	20.75	
VI	90.5	124-125	C <sub>6</sub> H <sub>6</sub>	77.37	77.09	7.57	7.64			6

<sup>a</sup> Where the reaction products are reasonably pure, crude yields are reported; otherwise yields are for once purified products. <sup>b</sup> B.p. 39-50°.

The infrared spectra of the bases and salts did not show any strong absorption in the C=N or in C=NH region and hence were of little diagnostic value.

At least two pathways are possible for the cyclization. First and the more obvious one involves the primary formation of a nitroso or hydroxylamino function from the amino group followed by a cyclization process on the  $\alpha$ -methylene carbon of the heterocyclic moiety. This pathway is indicated by the isolation of cyclic products in small amounts during the reduction of the nitro compounds in strong acid. Moreover, peracetic acid has been used recently for the oxidation of aromatic amines

to nitroso compounds.<sup>10</sup> Steric factors may play an important role since attempts to oxidize N,N-dimethyl - *o* - phenylenediamine and N,N - di(hydroxyethyl) - *o* - phenylenediamine were unsuccessful and rapid decomposition appeared to occur. The presence of a ring may fix the methylene function in close proximity to the nitroso group thereby helping the cyclization reaction to be realized.

An alternative mechanism involving the formation of an N→O bond of the cyclic tertiary amine followed

(10) R. R. Holmes and R. P. Bayer, *J. Am. Chem. Soc.*, **82**, 3454 (1960).

TABLE IV  
 ULTRAVIOLET SPECTRA OF CYCLIC PRODUCTS<sup>a</sup>

Compound	$\lambda_{\max}$ , m $\mu$			-log $E_{\max}$		
I	252	276	282	3.826	3.782	3.814
II	252	274	282	3.842	3.778	3.795
III	249	275	282	3.760	3.748	3.757
IV	256	284	292	3.658	3.677	3.677
V	255	284	292	3.685	3.662	3.653
VI	250	284	292	3.447	3.542	3.534
VII	254	282	288	3.659	3.866	3.869
VIII	255	283	288	3.921	3.866	3.869
IX	250	282	288	3.628	3.616	3.633
X	242			4.177		
XI	241			4.292		
XII	238			4.227		
XIII	252	275	282	3.869	3.796	3.788

<sup>a</sup> Ultraviolet spectra were determined in absolute ethanol with a Cary automatic recording spectrophotometer

by rearrangement to the carbinolamine, cyclization and oxidation seems unlikely. N-(Benzamidophenyl)-piperidine with peroxytrifluoroacetic acid was not oxidized under conditions normally employed for the oxidative cyclization. However, when the reaction was carried out for a longer period of time, a small yield of 1,2-tetramethylenebenzimidazole resulted. Hydrolysis was shown not to occur under the conditions of the experiment. N,N-Diacetylaminophenylpiperidine was also oxidized to the same tricyclic product.

**Acknowledgments.**—The authors are indebted to the A. P. Sloan Foundation for financial support which made this investigation possible. They thank Mr. P. E. McMahon for the infrared spectra, Miss C. Juan for the ultraviolet spectra and Mr. Josef Nemeth, Mrs. A. S. Bay and Miss Jane Liu for the microanalyses.

### Experimental

**Condensation of 2-Nitrochlorobenzenes with Piperidine, Pyrrolidine, Morpholine and Hexamethyleneimine.**—N-(2-Nitrophenyl)-piperidine,<sup>11</sup> N-(2-nitro-4-chlorophenyl)-piperidine, N-(2,4-dinitrophenyl)-piperidine,<sup>11</sup> N-(2-nitrophenyl)-morpholine,<sup>12</sup> N-(2-nitro-4-chlorophenyl)-morpholine,<sup>13</sup> N-(2,4-dinitrophenyl)-morpholine<sup>13</sup> and N-(2-nitrophenyl)-hexamethyleneimine<sup>6</sup> were prepared by the condensation of the appropriate halogen compound with the tertiary amine.

**Procedure A.**—A mixture of the halogenonitro compound with a threefold excess of the heterocyclic base was heated in an oil-bath at 90–100° for 1–2 hr. The resulting red solution or solid was cooled, poured into cracked ice, and stirred. The crystalline product was washed with water and recrystallized from dilute ethanol. If the product failed to crystallize even on cooling for several hours, the resulting oil was washed with water, the aqueous layer extracted with ether, the ether layer added to the separated oil, washed with water, dried and evaporated. Distillation yielded the pure material.

**Procedure B.**—The halogenonitro compound was dissolved in 95% ethanol and the heterocyclic base (4 mole equiv.) added in small quantities. The mixture was cooled to control any exothermic reaction. After the addition was complete, the mixture was heated on the steam-bath for 30 min. to 1 hr.; most of the ethanol was removed under reduced pressure and the product isolated as in A.

**Reduction of Nitro Compounds to Aromatic Amines.**—N-(2-Aminophenyl)-piperidine,<sup>9</sup> N-(2-amino-4-chlorophenyl)-piperidine,<sup>6</sup> N-(2-amino-4-nitrophenyl)-piperidine,<sup>6</sup> N-(2-amino-4-nitrophenyl)-morpholine,<sup>12</sup> N-(2-amino-4-chlorophenyl)-morpholine,<sup>6</sup> N-(2-amino-4-nitrophenyl)-morpholine<sup>9</sup> and N-(2-aminophenyl)-hexamethyleneimine<sup>6</sup> were prepared by reduction of the nitro compounds by various procedures.

**Procedure A.**—To a stirred mixture of 100 ml. of 0.78 N aqueous ammonium chloride and 30.0 g. of iron filings on a steam-cone was added in small quantities 10.0 g. of the nitro compound. The stirring was continued for 2 hr., the slurry filtered through Super-Cel while hot, and the residue washed with chloroform. The filtrate was extracted with chloroform, the combined organic solvent washed with water, dried and evaporated to leave a solid or brown oil. Purification was effected by recrystallization or by distillation in high vacuum.

**Procedure B.**—A solution of 10.0 g. of the nitro compound in 100 ml. of 95% ethanol was hydrogenated at 40 p.s.i. in the presence of 400 mg. of platinum oxide catalyst. When the theoretical amount of hydrogen has been taken up (usually in 0.5 hour or less), the solution was filtered from the catalyst, and the solvent removed under reduced pressure. The product was purified by recrystallization or distillation.

**Procedure C.**—To a mixture of 10 g. of nitro compound and 16 g. of granulated tin in a 500-ml. round-bottomed flask was added with shaking and cooling, 16 ml. of concentrated hydrochloric acid. After a few minutes vigorous reaction set in. More acid (20 ml.) was added and the flask heated on the steam-cone for 40 min. The flask was cooled, the contents carefully basified with aqueous sodium hydroxide, and the mixture extracted with ether. The slurry was filtered through Super-Cel, the ether layer separated, washed and dried. Evaporation yielded the crude amine.

**Procedure D.**—Partial reduction using sodium sulfide and powdered sulfur in ethanol was carried out according to the method of Saunders.<sup>6</sup>

**Procedure E.**—Employed reduction with iron and hydrochloric acid according to the method of Saunders.<sup>6</sup>

**N-(2-Benzamidophenyl)-piperidine.**—To a mixture of 1.2 g. of N-(2-aminophenyl)-piperidine and 10 ml. of 10% aqueous sodium hydroxide was added 1 ml. of benzoyl chloride. The mixture was shaken occasionally. When the odor of benzoyl chloride had disappeared, the solution was poured into cracked ice and water. The oil that separated was taken up in 95% ethanol and water added to turbidity. Cooling in the ice-box caused precipitation of a white crystalline solid. It was recrystallized from dilute ethanol; m.p. 79–80°, yield 1.6 g. (83%).

*Anal.* Calcd. for C<sub>18</sub>H<sub>20</sub>N<sub>2</sub>O: C, 77.10; H, 7.19; N, 9.99. Found: C, 77.30; H, 7.10; N, 9.81.

**N-(2-N',N'-Diacetylaminophenyl)-piperidine.**—A solution of 0.9 g. of the N-(2-aminophenyl)-piperidine in 4 ml. of acetic anhydride was heated under reflux for 1.5 hr. after which most of the solvent was removed in vacuum, and the residue diluted and basified with 10% aqueous sodium hydroxide. The solution was extracted with ether, the extract washed, dried and evaporated to give a pale yellow oil. On scratching with a few drops of 95% ethanol it crystallized to give needles, m.p. 76–78°.

*Anal.* Calcd. for C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 69.20; H, 7.74. Found: C, 68.92; H, 7.96.

**Oxidative Cyclization. General Procedure A.**—To a solution of 1 g. of aromatic amine (1 mole equiv.) in 20 ml. of purified methylene chloride was added 6 ml. (10 mole equiv.) of trifluoroacetic acid. The mixture was stirred and 3 ml. (5 mole equiv.) of 30% hydrogen peroxide added dropwise. An exothermic reaction took place and the color darkened. After addition was complete, the solution was stirred under reflux on the steam-cone for 15–30 min. during which time the color gradually faded. The solution was cooled, the organic solvent washed with aqueous sodium carbonate and with water, dried (sodium sulfate) and evaporated under reduced pressure to give an oil which usually crystallized on cooling. Purification was accomplished by distillation in high vacuum followed by recrystallization or by chromatography on neutral alumina followed by recrystallization or sublimation.

**Procedure B.**—To a solution of 1.0 g. of the amine in 6 ml. of trifluoroacetic acid was added dropwise with stirring 3 ml. of 30% hydrogen peroxide. The mixture turned dark in color. When the exothermic reaction had subsided,

(11) M. K. Seikel, *J. Am. Chem. Soc.*, **62**, 750 (1940).

(12) C. B. Kremer, M. Meltsner and L. Greenstein, *ibid.*, **61**, 2552 (1939).

(13) R. H. Harradence and F. Lions, *J. Proc. Roy. Soc., N.S.W.*, **70**, 406 (1937).

the solution was heated on the steam-cone for 15–30 min. During this time the color faded to yellow. Most of the excess acid was removed under reduced pressure, the residue poured into cracked ice, basified with aqueous sodium carbonate solution and extracted with chloroform. The extract was washed, dried and evaporated.

**Oxidation of N-(2-Benzamidophenyl)-piperidine with Peroxytrifluoroacetic Acid.**—To a stirred solution of 650 mg. of N-(2-benzamidophenyl)-piperidine in 4 ml. of trifluoroacetic acid was added 2 ml. of 30% hydrogen peroxide.

No reaction was apparent. On heating on the steam-cone with stirring, the color of the solution turned brown and on continued heating for 1 hour a yellow solution resulted. This was worked up as usual to give the crystalline piperidinebenzimidazole, m.p. 96–98°, yield 140 mg. (35%).

**Oxidation of N-(2-N',N'-Diacetylaminophenyl)-piperidine.**—Oxidation of N-(diacetylaminophenyl)-piperidine with peroxytrifluoroacetic acid as described above yielded 120 mg. (28%) of crystalline piperidinebenzimidazole, m.p. 98–99°.

CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY 4, CALIFORNIA]

## Adrenocorticotropin (ACTH). XXIII. A Sedimentation Study of the State of Aggregation of Ovine Pituitary ACTH in Acidic and Basic Solutions

BY PHIL G. SQUIRE AND CHOH HAO LI

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The state of aggregation of ACTH in solution has been the subject of very few quantitative investigations, although suggestions have been advanced that the hormone forms soluble association products near the region of insolubility ( $pH$  6–8) and in basic media. We have studied the sedimentation behavior of ACTH by the sedimentation velocity and sedimentation equilibrium methods in three buffers: (I) HCl-KCl,  $pH$  1.3, ionic strength 0.200; (II) formic acid-formate, NaCl,  $pH$  3.5, ionic strength 0.200; and (III) carbonate-bicarbonate,  $pH$  10.1, ionic strength 0.100. Correction for charge effects was made when necessary and duplicate sedimentation equilibrium experiments at different initial concentration served to distinguish between reversible association and simple heterogeneity. Diffusion coefficients were calculated from data obtained during the approach to equilibrium and from boundary spreading in sedimentation velocity experiments. In buffer I, the dependence of sedimentation and diffusion coefficients and molecular weight ( $M$ ) upon concentration is slight, and all 3 parameters correspond to the monomer ( $M = 4540$ ). Molecular frictional ratios calculated from  $s$  and  $D$  indicate that ACTH assumes a highly extended configuration at  $pH$  1.3. In buffer II, evidence of reversible association yielding tetramers (or higher) was found. The first association constant (dimer formation) was found to be 0.33 on the g./100 ml. concentration scale. Evidence is presented that the successive association constants increase in magnitude. In buffer III, soluble aggregates (tetramers) not in equilibrium with monomer were detected. This and evidence from other experiments suggests that ACTH undergoes irreversible association in a basic medium.

Extensive chemical studies<sup>1,2</sup> have established the complete amino acid sequence of ovine pituitary adrenocorticotrophic hormone ( $\alpha_s$ -ACTH); consequently the molecular weight of the unit linked by covalent bonds is known with a high degree of certainty to be 4540 at its isoionic point. Little is known, however, of the state of aggregation of the molecular kinetic unit in solution. Brown, *et al.*,<sup>3</sup> determined the molecular weight of porcine pituitary ACTH by means of the Archibald procedure in a solution containing 0.05  $M$  KCl and 0.05  $M$  HCl per liter and reported that except for a trace of an impurity of high molecular weight, which was detectable in both the Archibald and synthetic boundary cell experiments, the preparations appeared homogeneous, and the molecular weight calculated from these experiments was in good agreement with that calculated from the amino acid content (4567).

Except for a brief summary<sup>2</sup> of the results of some preliminary sedimentation and diffusion studies, the molecular kinetic behavior of highly purified ovine ACTH has not been reported. The molecular weight calculated from these studies which were carried out in a 0.100  $M$  HCl, 0.200  $M$  KCl solvent, also was in good agreement with the value calculated from the amino acid composition.

Both studies were performed in the very low  $pH$  range because of the insolubility of the hormone in the neighborhood of neutrality and also because of

a rather widely held but experimentally unconfirmed notion that ACTH tends to aggregate when the limiting solubility is approached. It may be that the basis for this contention is largely reasoning by analogy from the rather extensive studies of the association of insulin which has a similar solubility behavior.

Another suggestion that ACTH forms soluble association products was derived from the observation<sup>4</sup> that  $\alpha_s$ -ACTH, which dialyzes quite readily in acidic media, is non-dialyzable when dialyzed against dilute ammonia. This suggested that the non-dializability of the hormone in alkaline solution was due to the formation of soluble aggregates.

The object of the investigation to be reported<sup>5</sup> here was to provide a physicochemical characterization of the hormone by means of sedimentation velocity and sedimentation equilibrium experiments in strongly acidic media where intermolecular association was not expected and to compare these results with those obtained in buffers where association has been postulated, and to provide, if possible, a quantitative description of the association reactions involved. To a very large degree, the course and extent of this investigation has been determined by the scarcity of ACTH polypeptide. The amount required for this study was 25 mg.

### Theoretical

Analysis of the sedimentation equilibrium experiments was based largely on the equation of

(1) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 686 (1955).

(2) C. H. Li, *Advances in Protein Chem.*, **11**, 101 (1956).

(3) R. A. Brown, M. Davies, M. Englert and H. R. Cox, *J. Am. Chem. Soc.*, **78**, 5077 (1956).

(4) C. H. Li, *Bull. Soc. Chem. Biol.*, **40**, 1757 (1958).

(5) A brief report of this work was presented at the 138th meeting of the American Chemical Society, New York, September 1960.