Dr. Eric G. Snyder for the crude streptomycin salts used. The assistance of Messrs. L. G. Colio and M. Kuna with the assays and Mr. S. V. Lieberman in preparing aluminas is gratefully acknowledged.

Summary

A method which is adaptable to large quantities of material has been proposed and evaluated for increasing the activity of crude streptomycin preparations by partial adsorption on alumina. From initial potencies in the region of 300 γ/mg. increases up to 600 units per mg. were readily obtained by single or multiple agitations of a methanolic solution of the antibiotic with acid-washed alumina.

PHILADELPHIA, PENNSYLVANIA RECEIVED JULY 31, 1946

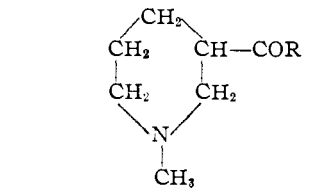
[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Rearrangements between Primary Ethanolamides of Carboxylic Acids and the Corresponding Aminoethylesters*

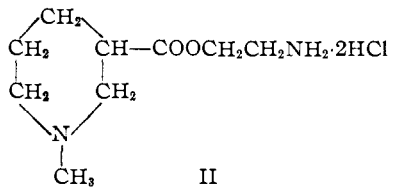
BY ARTHUR P. PHILLIPS AND RICHARD BALTZLY

While it is well known that esters of β-amino alcohols, especially those with primary amino groups, tend to rearrange to ethanolamides and it has been reported that the latter are in turn transformed to salts of aminoesters by hydrogen chloride,[1] the mechanism of these rearrangements and the exact conditions required remain undefined.

In connection with a problem of pharmacological interest, the ethanolamides of certain carboxylic acids were desired. It seemed best to prepare these by heating the corresponding esters with excess ethanolamine, using the latter both as reactant and high-boiling solvent.[2] Pertinent data for the ethanolamides thus prepared are presented in Table I.



I, R = NH—CH_2CH_2OH
Ia, R = OCH_3



II

TABLE I

ETHANOLAMIDES, $RCONHCH_2CH_2OH$

R =	Ratio of moles ethanolamine to moles ester	Reflux time, hours	Yield, %	B. p., °C.	Mm.	M. p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3-(N-Methyl)-hexahydropyridyl	6	2	100	183–185	1	.....	58.03	57.74	9.75[a]	9.83
3-Pyridyl	1.5	2	92–95	210–212	2	89–90	57.80	57.89	6.08	5.85
4-Pyridyl	5	1/2	90–95	220	1	134–135	57.80	57.83	6.08	6.10
4-Quinolyl	5	2	95	.....		112–113	66.84	66.69	5.60	5.92
4-Hydroxy-3-quinolyl	12	3/4	100	.....		253–254	62.04	62.05	5.21	5.24
Phenyl	5	1	95–100	185–187	1	60–61	65.41	65.33	6.72	6.64
2-Hydroxyphenyl	2.5	1/2	100	210–215	2	113–114	59.64	59.42	6.13	6.07
Benzyl	5	2	100	202–204	1	60–61	67.00	67.14	7.31	7.04
n-Propyl	8	3 1/2	100	150–151	1	.....	54.92	54.72	10.00	10.00

[a] Neutral equivalent: calcd., 186.1. Found: 186.1, 186.2.

In the first attempt to prepare the ethanolamide (I) from dihydroarecoline (Ia) a variation in technique produced an unexpected result. After removal of ethanolamine *in vacuo*, the residual, high-boiling, viscous oil was taken up in ethanol and treated with an excess of ethanolic hydrogen chloride in an attempt to isolate the amide as its hydrochloride. The product, however, was shown by analysis to be the dihydrochloride of a substance isomeric with the ethanolamide. This substance can hardly be formulated as other than the dihydrochloride of the aminoethyl ester (II).

The dihydrochloride was stable during recrys-

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(1) (a) Immediata and Day, *J. Org. Chem.*, **5**, 512 (1940); (b) Kanao, *J. Pharm. Soc. Japan*, **48**, 1070 (1928); (c) Cope and Hancock, THIS JOURNAL, **66**, 1448, 1453, 1738 (1944); (d) Reasenberg and Goldberg, *ibid.*, **67**, 933 (1945).

(2) *Cf.* D'Alelio and Reid, *ibid.*, **59**, 111 (1937); Wenker, *ibid.*, **57**, 1079 (1935). Alternative preparations are given by Knorr and Rössler, *Ber.*, **36**, 1278 (1903); Fränkel and Cornelius, *ibid.*, **51**, 1657 (1918).

TABLE II

AMINOETHYL ESTER HYDROCHLORIDES, $RCOOCH_2CH_2NH_2 \cdot HCl$

R[a]	Compound	Yield, %	M. p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Chlorine Calcd.	Chlorine Found
3-(N-Methyl)-hexahydropyridyl	II	35[b]	213–214	41.67	41.71	7.77	7.93	27.39	27.43
3-Pyridyl	III	65[b]	213–214	40.15	40.56	5.06	5.18	29.71	29.57
4-Pyridyl	IV	65[b]	213–214	40.15	40.50	5.06	5.00	...	...
4-Quinolyl	V	55[b]	205–206	49.81	49.66	4.88	4.93	...	...
Phenyl	VI	65[c]	142–143	53.57	53.24	6.00	5.94	...	...
2-Hydroxyphenyl	VII	20[c]	189–190	49.63	49.78	5.56	5.46	...	...

[a] One additional mole of hydrogen chloride was bound by the heterocyclic nitrogen in compounds II–V. [b] Crystallized from alcohol–acetone. [c] Crystallized from alcohol–acetone–ether.

tallization and drying and dissolved readily in water giving a solution of *p*H 5. When, however, two equivalents of alkali were added and the solution was returned to *p*H 5 by back-titration with hydrochloric acid, only one equivalent of acid was required (Fig. 1), indicating the loss of one basic group in this treatment.

Further investigation showed that when the product of the ester–ethanolamine reaction was isolated directly without acid treatment the ethanolamide was obtained in every case. The ethanolamides, however, when treated with absolute ethanolic hydrogen chloride either with heating or at room temperature, were transformed to a considerable extent into the hydrochlorides of the isomeric aminoethyl esters. The latter, in turn, on addition of an amount of alkali sufficient to neutralize the bound hydrogen chloride, followed by back-titration with standard acid, were found to have lost one basic group.

By the process outlined above the aminoethyl esters listed in Table II have been prepared from the corresponding ethanolamides. All these aminoesters have been shown to undergo the reverse rearrangement almost instantaneously and in quantitative fashion. The ethanolamide of phenylacetic acid, when warmed with ethanolic hydrogen chloride, yielded no isolable amount of salt-like material but gave instead a mixture of ethyl phenylacetate and another substance, probably chloroethyl phenylacetamide.

While no definite conclusions as to mechanism could be drawn from the experiments reported in the literature, it appeared probable that these reactions were intramolecular and a possible intermediate was apparent in the family of oxazolines studied by Gabriel and Heymann.[3] These authors had, in fact, prepared aminoethyl esters indirectly from the corresponding bromoethylamides by the series of transformations A–C.

$$RCONHCH_2CH_2Br \xrightarrow{KOH\ (1\ eq.)} \underset{A}{} \qquad \underset{B}{R-C(=N-CH_2)(-O-CH_2)} \xrightarrow[1\ eq.HCl]{warm\ with} R-COOCH_2CH_2NH_2 \cdot HCl \quad (C)$$

(3) Gabriel and Heymann, *Ber.*, **23**, 2493 (1890).

The oxazolines (B) were feebly basic oils, sufficiently stable under neutral or alkaline conditions to be steam-distilled. They have also been prepared from ethanolamides by drastic dehydration (*e. g.*, by the action of phosphorus pentoxide or by heating at a high temperature). When warmed with aqueous acids the ring is opened and salts of aminoethyl esters result.

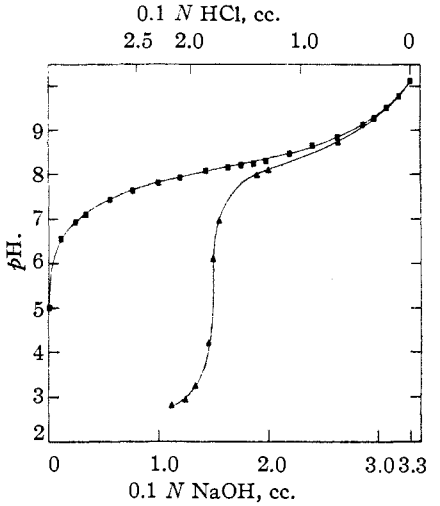


Fig. 1.—Titration curve of N-methylhexahydronicotinic acid amino ethyl ester dihydrochloride (0.159 millimole): ■, points on curve of direct titration; ▲, points on curve of back-titration.

Since the rearrangements as applied to derivatives of benzoic acid involve changes between basic and non-basic substances and since 2-phenyloxazoline was already known,[3] the benzoic ethanolamide–aminoethylbenzoate system seemed most suitable for more detailed study.

For forward change, from ethanolamide to aminoethyl ester, does not occur to any appreciable extent in dilute aqueous hydrochloric acid. In absolute ethanolic hydrogen chloride solution, the change is relatively slow and is not quantitative. After crystallization of benzoic acid aminoethylester hydrochloride, it was possible to isolate from the mother liquors a picrate whose properties agreed with those recorded for 2-phenyloxazoline picrate and whose analysis corresponded to the required composition.

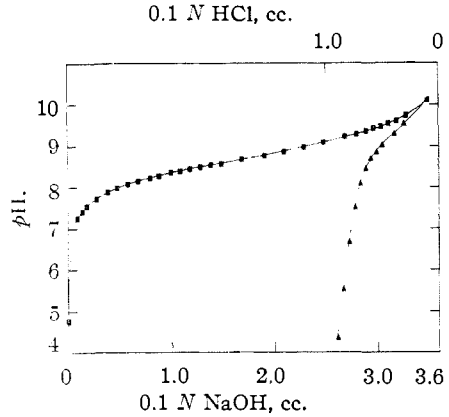


Fig. 2.—Titration curve of aminoethyl benzoate hydrochloride (0.3 millimole): ■, points on curve of direct titration; ▲, points on curve of back-titration.

The reverse change, on the other hand, is extremely rapid. When taken to *p*H 10 about 95% of the ester base disappears within two minutes (see Fig. 2). The results of titrations of aminoethylbenzoate hydrochloride and of the other amino ester salts of Table II are presented in Table III. "Neutral equivalents" were determined by addition of excess alkali to the aqueous solutions of the salts, followed by back-titration to the original *p*H. A rapid and quantitative reverse rearrangement is essential if good "neutral equivalents" are to be obtained by this method, since it is the loss of acid-binding power during the process that is being measured. Conversely, the approximation of the observed "neutral equivalent" to the calculated value is a measure of the speed and completeness of the rearrangement. Although in most cases the agreement between theoretical and experimental values was excellent, the derivatives of benzoic and salicylic acids gave marked deviations when the titrations were completed rapidly. With aminoethylbenzoate hydrochloride it was found that the deviation dropped from 6 to 2% when the solution was allowed to stand twenty minutes after addition of the alkali and before the back-titration. Longer standing with alkali did not affect the result but in electrometric titrations there was some indication of increased buffer concentration. This suggests that when the rearrangement is relatively slow the situation may be complicated by saponification.

TABLE III

TITRATIONS OF AMINOETHYL ESTER HYDROCHLORIDES

Compd. no.	Time elapsed during titration, minutes	"Neutral equivalent" Calcd.	Found
II	2	259.2	259.6
III	2	239.1	237.8
IV	2	239.1	240.2
V	2	289.1	290.8
VI	2	201.5	215
VI	2	201.5	214
VI	20[a]	201.5	206
VI	90[b]	201.5	206
VII	10	217.6	228.8

[a] Solution allowed to stand twenty minutes prior to back-titration. [b] Solution allowed to stand ninety minutes prior to back-titration.

After titrations of aminoethylbenzoate hydrochloride, benzoic ethanolamide was demonstrated by nearly quantitative recovery as its phenyl urethan, but no detectable amounts of aminoethylbenzoate or 2-phenyloxazoline were present. Since the latter is fairly stable under neutral or alkaline conditions it is inconceivable that it could mediate the reverse rearrangement.

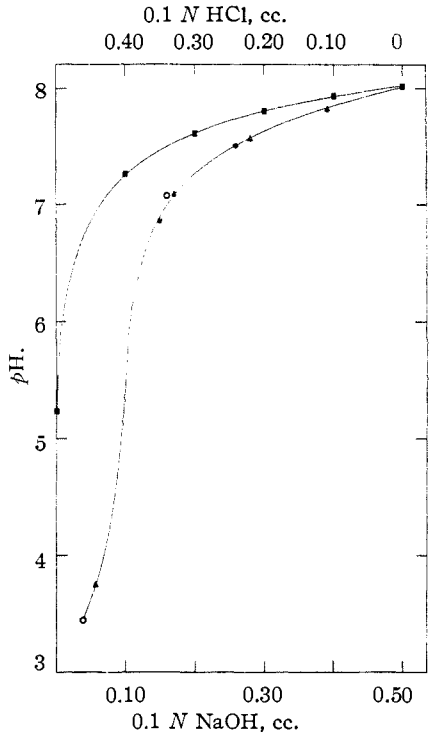
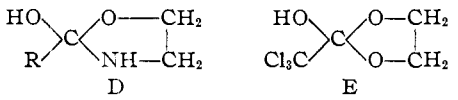


Fig. 3.—Titration curve of aminoethyl benzoate hydrochloride (0.25 millimole): ■, points on curve of first direct titration; ▲, points on curve of first back-titration; ●, points on curve of second direct titration; ⊙, points on curve of second back-titration.

Examination of the curves of a partial titration (Fig. 3) shows that rearrangement does not proceed at an appreciable rate below *p*H 7.5. Between *p*H 7.5 and 8, significant rearrangement takes place, but apparently only about one-fifth of the base that should have been liberated is found to be lost. Since, when the *p*H is taken to 9.5–10, more than 95% rearrangement is observed after standing two minutes, it seems clear that the process is a function of *p*H not merely through liberation of the base.

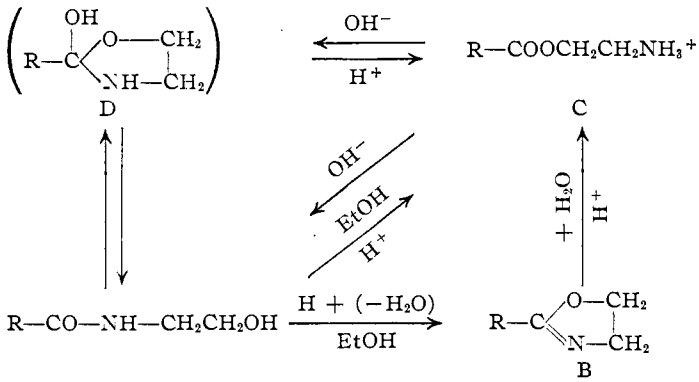
The oxazoline type being definitely excluded as the intermediate in the reverse change although

possible in the forward one, another intermediate (D) is suggested, analogous to the cyclic forms of the sugars. A similar structure (E) was demonstrated by Meerwein and Sönke,[4] to be present as a

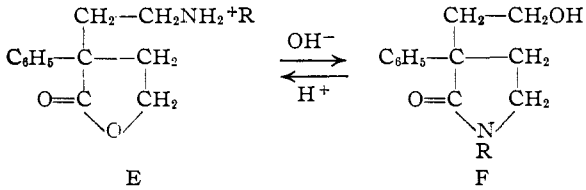


D E

tautomeric form in glycol mono-trichloroacetate. A substance of type D would be expected to be extremely labile (and correspondingly hard to demonstrate). Meerwein proved the existence of E by its reaction with diazomethane. The substance D (R = C_6H_5), if present as a tautomer in solutions of benzoic ethanolamide, does not react with diazomethane to give a product capable of forming an insoluble picrate. While this intermediate is so far hypothetical we feel it is still the most probable and consider that the general scheme of the rearrangement is best formulated as shown.



A further reason for considering the oxazoline to be the result of a side reaction and not a true intermediate in the forward rearrangement is that such a substance could not be formed in the related transformations of secondary ethanolamides[1d] where the aminoesters are relatively more favored. While we consider it unsound to assume that similar processes should always proceed by one only of two alternate mechanisms, both of which should be capable of giving the observed result, there is no doubt that the argument by analogy in this case is very strong. A rearrangement of essentially the same type was discovered by Walton and Green[5a] lactones of the type E passing reversibly into lactams of type F. Dr. Walton[5b] has pointed out that in this case

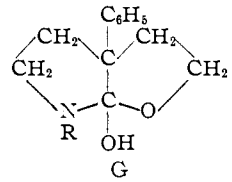


E F

(4) Meerwein and Sönke, *Ber.*, **64**, 2375 (1931).

(5) (a) Walton and Green, *J. Chem. Soc.*, 315 (1945); (b) private communication.

also a ring-chain tautomeric intermediate G can be formulated.



G

Experimental

Preparation of the Ethanolamides.—The appropriate ester was mixed with 2–12 moles of ethanolamine (the larger proportions being used only where the ester was itself low-boiling or else a high-melting solid with low solubility) usually giving a two-phase suspension at room temperature. The mixture (which became homogeneous on heating) was refluxed (metal bath) for the times indicated in Table I. By following the temperature of the refluxing mixture it was usually possible to determine when the reaction was complete. In most cases reaction appeared to be over in ten to fifteen minutes but refluxing was continued for the longer periods specified. The excess ethanolamine was largely removed by distillation *in vacuo* and purification was accomplished by vacuum distillation or by crystallization.

The purified amides were identified by analysis for carbon and hydrogen, and titrations were performed, using methyl red as indicator, to determine the presence or absence of any basic groups. In no case was there any indication of material having a base strength of the same order of magnitude as ammonia, except with the N-methylpiperidine derivative, and in this instance precisely one equivalent of strong base was found.

Although the purified amides showed no basic matter present, in a number of cases titrations performed on the amides as first isolated by vacuum distillation showed that a very small amount of some strong basic substance was obtained. An attempt was made to isolate the base in one case.

Isolation of Basic Material from *n*-Butyric Ethanolamide.—Treatment of a 2-g. sample of crude *n*-butyric ethanolamide (once distilled *in vacuo*) with an excess of alcoholic picric acid solution gave 0.2 g. of a picrate, which after recrystallization from alcohol formed yellow, diamond-shaped prisms; m. p. (dec.) 216–217°.

Anal. Calcd. for $C_8H_8O_7N_4$: C, 35.29; H, 2.98; N, 20.59. Found: C, 34.98; H, 2.96; N, 20.22.

The analytical data fit the empirical formula: $(CH_2{=}CHNH_2HOC_6H_2(NO_2)_3)_x$. The tetrapicrate of bis-aminoethylpiperazine[6] is recorded as forming yellow leaflets, m. p. 220°, and it is at least a reasonable possibility that this piperazine compound might be formed in small amounts in refluxing ethanolamine.

Assuming the basic substance to be bis-aminoethylpiperazine, the yield of picrate corresponds to the presence of 1.6% of the crude *n*-butyric ethanolamide. A titration of 44.7 mg. of this ethanolamide with 0.109 *N* hydrochloric acid to the methyl red end-point required 0.20 cc., corresponding to the presence of 2.1% of the bis-aminoethylpiperazine. This basic substance was easily removed from the ethanolamide on further purification.

Preparation of the Aminoethylester Hydrochlorides.—In general the ethanolamides (0.02–0.05 mole) were dissolved in about 50 cc. of absolute ethanol containing 4–5 equivalents of hydrogen chloride. The solutions were heated on the steam-bath one-half to two hours, some of the alcohol being allowed to evaporate. In some cases the crystalline hydrochlorides separated on cooling, in others acetone or ether was added to the point of incipient turbidity.

(6) Franchimont and Kramer, *Rec. trav. chim.*, **31**, 40 (1912).

Aminoethylbenzoate hydrochloride was also prepared by dissolving 0.01 mole of benzoic ethanolamide in 15 cc. of absolute ethanol containing 0.05 mole of hydrogen chloride. To this was added absolute ether to the point of incipient turbidity and the solution was allowed to stand at room temperature. An oil separated which crystallized on the third day. At the end of a week the solid was collected and the mother liquor examined for the presence of oxazoline. The yields were not significantly different from those in the parallel experiment at steam-bath temperature.

The mother liquors from the aminoethylbenzoate hydrochloride were examined for the presence of other basic material by fractional precipitation with picric acid. It was possible to obtain in 30% yield a substance crystallizing in yellow needles, m. p. 178–179°. Gabriel and Heymann[3] give 177° as the melting point of 2-phenyloxazoline picrate.

Anal. Calcd. for $C_9H_9ON \cdot C_6H_3O_7N_3$: C, 47.86; H, 3.22. Found: C, 48.09; H, 3.40.

In an attempt to prepare the aminoethylbenzoate hydrochloride with aqueous hydrochloric acid an aqueous solution of 80.4 mg. of benzoic acid ethanolamide (vol., 10 cc.) was treated with 5.00 cc. of 0.109 *N* hydrochloric acid and allowed to stand for four hours at room temperature. Back-titration to the methyl red end-point required 4.72 cc. of 0.112 *N* sodium hydroxide. This corresponds to a loss of hydrochloric acid of from 2.5–3% and indicates that the forward change (amide to aminoester) either proceeds not at all or to an insignificant extent under these conditions. This is in marked contrast to the speed and extent of the reverse change under comparable conditions.

When subjected to the usual reaction conditions the ethanolamide of phenylacetic acid gave neither aminoester hydrochloride nor any basic substance forming an insoluble picrate. There was obtained in 20–30% yield a substance melting at 82–83° and containing organically bound halogen. It appears likely that this was the chloroethylamide of phenylacetic acid, slightly contaminated with phenylacetic ethanolamide.

Anal. Calcd. for $C_{10}H_{12}ONCl$: C, 60.76; H, 6.08. Found: C, 61.53; H, 5.92.

There was also obtained in 50–60% yield an oil boiling at 224° and presumably ethyl phenylacetate whose b. p. is given as 226°.[7]

Titrations of Aminoethylester Hydrochlorides.—Weighed samples of the aminoethyl ester hydrochlorides were dissolved in water and to these solutions were added measured amounts of 0.1 *N* sodium hydroxide calculated to be slightly in excess of these required to neutralize the hydrochloric acid bound in the salts. The solutions were the back-titrated with 0.1 *N* hydrochloric acid during the elapsed times given in Table III. With esters II, VI and VII methyl red was used as the indicator for the back-titration and the results showed the loss of one basic group through rearrangement in each case. "Neutral equivalents" (same as molecular weights, see Table III) were calculated from the titration data using as the volume of alkali equivalent to the aminoester salt the difference between the total alkali added and the amount of acid required to return to the original *p*H (or to the particular indicator end-point selected).

With esters III, IV and V containing heterocyclic aromatic rings, phenol red was chosen as the indicator for the back-titration as it had previously been found that bases of the order of strength of ammonia could be titrated successfully in the presence of 15–20 equivalents of pyridine or quinoline by use of this indicator. The loss of two basic groups was shown with these latter compounds, the side chain one by rearrangement, and the nuclear one by choice of indicator. In these instances the "neutral equivalents" were one-half the molecular weights, and were multiplied by two for inclusion in Table III.

Electrometric titrations of the esters II and VI are shown in Figs. 1 and 2, respectively. Further details as to the rearrangement reaction were obtained by other electrometric titrations examining the behavior in the region corresponding to the first portion of Fig. 2 (shown in Fig. 3).

Action of Picric Acid on the Various Derivatives of Benzoic Acid.—Both the aminoethyl ester hydrochloride, VI, and 2-phenyloxazoline in dilute aqueous acid solutions, when treated with a saturated aqueous picric acid solution, gave immediate and quantitative precipitation of the picrates of the respective bases. Benzoic acid ethanolamide gave no precipitate with the picric acid solution under similar conditions. When aminoethylbenzoate hydrochloride was treated with excess alkali and immediately returned to *p*H 4 with dilute hydrochloric acid, the resulting solution gave no precipitate with the picric acid solution. These facts make it clear that even within one minute (or less) of the addition of excess alkali to the aminoester salt neither aminoester nor oxazoline can be present thus proving it impossible to liberate the former base as such, and completely eliminating the latter as an intermediate in the reverse rearrangement.

Isolation of Benzoic Ethanolamide as the Product of Neutralization.—Five-tenths gram of aminoester hydrochloride was dissolved in 5 cc. of water, 25 cc. of 0.1 *N* sodium hydroxide solution was added and the mixture was allowed to stand fifteen minutes. The water was then removed *in vacuo* at 40° and the residue was taken up in acetone, filtered from sodium chloride and again evaporated *in vacuo*. The oily residue was dissolved in benzene and treated with 1 cc. of phenyl isocyanate. There was obtained 0.55 g. (80%) of the phenylurethan of benzoic ethanolamide, m. p. 195–197°, identical with that prepared directly from benzoic ethanolamide.

Acknowledgment.—The authors wish to express their gratitude to Mr. Samuel Blackman for the microanalyses here recorded and to Mr. Everett H. Lang for the electrometric titrations.

Summary

1. In the presence of alcoholic hydrogen chloride, the ethanolamides of a number of cyclic carboxylic acids have been found to rearrange to the corresponding aminoethylester hydrochlorides. This reaction may take place through cyclization to an intermediate oxazoline.

2. In aqueous solution above *p*H 7.5 the aminoethyl ester hydrochlorides were found to rearrange to ethanolamides almost instantaneously. The oxazoline cannot here be the intermediate and a ring–chain tautomeric form is suggested as mediating the rearrangement.

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(7) *Cf.* Elfeldt, *Ber.*, **24**, 3218 (1891), who indicates that 2-benzyloxazoline is hydrolyzed more easily than 2-phenyloxazoline.