is determined in the first stage, the variation in *P* arises in the second stage. Thus they propose the preceding mechanism for catalyzed esterification.

Summary

1. The study of the effect of polar groups has shown that the velocity of hydrion-catalyzed esterification of benzoic acids is increased by displacement of electrons toward the carboxyl group.
2. Good agreement was found between the experimental values of log *k* for the esterification and the values predicted by Hammett's equation.
3. It appears that the non-exponential factor of the Arrhenius equation as well as the activation energy increases upon passing from the rapidly esterified acids to those more slowly esterified.

BLOOMINGTON, INDIANA RECEIVED AUGUST 4, 1937

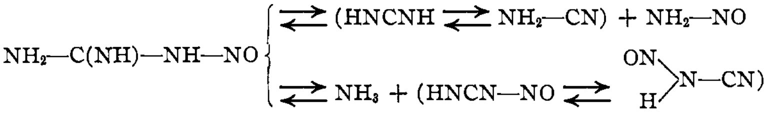
[CONTRIBUTION NO. 156 FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Studies in the Urea Series. XV.[1] Transformations of Nitrosoguanidine. Alkylnitrosoguanidines. N-R,N′-R′-Dialkylguanidines

BY TENNEY L. DAVIS AND EDWARD N. ROSENQUIST

It has been shown that nitroguanidine in aqueous solution dearranges in two modes,[2] to produce on the one hand cyanamide and nitroamide, on the other ammonia and nitrocyanamide, and advantage has been taken of the dearrangement for the preparation of guanidine carbonate by the action of ammonium carbonate on nitroguanidine, and of N-alkyl,N′-nitroguanidines[3] by the action of aliphatic amines. Nitrosoguanidine evidently dearranges in aqueous solution to produce cyanamide and nitrosoamide,[2] for the solution on boiling gives off nitrogen and yields dicyandiamide, and in the presence of mineral acids nitrosates certain aromatic compounds. Fuller, Lieber and Smith in a recent paper[4] have reported that nitrosoguanidine yields nitrogen when treated with sodium in liquid ammonia, and have taken the fact to be evidence that dearrangement occurs. In the present work we have studied the dearrangement of nitrosoguanidine and of several N-alkyl,N′-nitrosoguanidines procured by the reduction of the corresponding alkylnitroguanidines, and have been led to the preparation of alkylated guanidines containing different alkyl groups on two different nitrogen atoms. Very few substances of this type have been reported. Nitrosoguanidine and the N-alkyl,N′-nitrosoguanidines appear to dearrange to produce the unknown and unstable nitrosoamide.[5]

Corresponding to the two modes in which nitroguanidine dearranges, nitrosoguanidine might be expected to dearrange as follows.



$$\mathrm{NH_2{-}C(NH){-}NH{-}NO}\begin{cases} \rightleftarrows (\mathrm{HNCNH} \rightleftarrows \mathrm{NH_2{-}CN}) + \mathrm{NH_2{-}NO} \\ \rightleftarrows \mathrm{NH_3} + (\mathrm{HNCN{-}NO} \rightleftarrows \begin{matrix}\mathrm{ON}\\ \mathrm{H}\end{matrix}\!\!>\mathrm{N{-}CN}) \end{cases}$$

In the presence of ammonia the dearrangement is largely in the first of these modes. Refluxed with a large excess of ammonia water, nitrosoguanidine yields guanidine, melamine (the trimer of cyanamide), ammeline and ammelide (products formed either by the partial hydrolysis of melamine or by the mixed polymerization of cyanamide and cyanic acid), and a small quantity of urea. Repeated slow evaporation with ammonia water gives less guanidine and more ammeline, while refluxing with ammonium carbonate gives an excellent yield of guanidine carbonate comparable to that which has been obtained from nitroguanidine by similar treatment. The mass action effect of ammonia in these experiments might be expected to inhibit any dearrangement in the second of the above-indicated modes, but the production of a small amount of urea indicates that it probably occurs to some extent. Nitrosocyanamide

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[1] No. XIV of this series, THIS JOURNAL, **59**, 1993 (1937).

[2] Davis and Abrams, *Proc. Am. Acad. Arts Sci.*, **61**, 437 (1926).

[3] Davis and Luce, THIS JOURNAL, **49**, 2303 (1927); Davis and Elderfield, *ibid.*, **55**, 731 (1933).

[4] Fuller, Lieber and Smith, *ibid.*, **59**, 1150 (1937).

[5] Schwarz and Giese, *Ber.*, **67**, 1108 (1934), attempted to prepare nitrosoamide by the action of nitrosyl chloride on potassium amide in liquid ammonia. They observed a flash of color during the reaction and were able to show the formation of ammonium nitrite, water and nitrogen. They evidently had nitrosoamide momentarily, but the substance in the presence of ammonia was unstable even at the low temperature at which they worked.

would be expected to decompose into nitrogen and cyanic acid, and the cyanic acid would combine with ammonia to form urea.

In the presence of aliphatic amines nitrosoguanidine, unlike nitroguanidine, dearranges principally to produce cyanamide. This combines with the amines to form alkylated guanidines. When an excess of primary or secondary amine is used, the aqueous solution warms up slightly, gives off nitrogen, and in a few hours at room temperature loses its color and no longer contains any nitrosoguanidine. In this way we have prepared and isolated in the form of the picrates methyl-, ethyl-, *n*-butyl-, *n*-heptyl-, N,N-dimethyl-, benzyl-, and piperidoguanidines in yields varying from 15 to 36%. In the experiment with benzylamine, in addition to the benzylguanidine, a considerable amount of *sym*-dibenzylurea was obtained. The presence of this material may perhaps be explained by supposing that dearrangement occurred in the second of the above-indicated modes, and that the cyanic acid combined with the benzylamine to form benzylurea which by the usual dearrangement went over to *sym*-dibenzylurea. It seems less likely that the dibenzylurea was produced by the hydrolysis of the dibenzylguanidine.

In the presence of aniline or methylaniline nitrosoguanidine in boiling aqueous solution dearranges to produce cyanamide which, however, does not combine with the aromatic amine but dimerizes and yields dicyandiamide quantitatively. When nitrosoguanidine was allowed to stand in aqueous solution for three days at laboratory temperature with a large excess of aniline hydrochloride, 77% of it was recovered unchanged and a small amount of *p*-aminoazobenzene was isolated —indicating that a small amount of nitrous acid had been present.[6]

Since nitrosoguanidine does not react with primary and secondary aliphatic amines, in a manner analogous to that in which nitroguanidine reacts, to produce N-alkyl,N′-nitrosoguanidines, we have prepared several alkylnitrosoguanidines by the reduction of the corresponding nitroguanidine derivatives. In 1934 when these experiments were in progress, Sabetta, Himmelfarb and Smith[7] and ourselves worked out independently a method for the preparation of nitrosoguanidine by the reduction of nitroguanidine with zinc dust and aqueous ammonium chloride. They reported yields of 40 to 60%; we have secured yields of 45 to 52%. Since this method is much superior to the method by which Thiele[8] originally prepared nitrosoguanidine, we have applied it to the preparation of the alkylnitrosoguanidines. Using 50% alcohol as the reaction medium, keeping the mixture cold and evaporating slowly, we have procured N-methyl,N′-nitrosoguanidine, yellow needles, melting with decomposition at 93°. *n*-Butyl- and benzylnitrosoguanidines could not be obtained in the crystalline state but existed in the form of orange-red oils readily soluble in water. They were prepared by carrying out the zinc dust reduction in aqueous ammonia in order to avoid the presence of chloride ions, and were isolated and analyzed in the form of their silver salts. The silver salts, suspended in a mixture of ether and methyl alcohol and treated with hydrogen sulfide, gave silver sulfide and the orange-red oils again. The silver salts of the alkylnitrosoguanidines are white; they darken rapidly on exposure to light and explode violently when heated. The alkylnitrosoguanidines which we have studied are fairly unstable compounds; even the crystalline methyl compound after standing for a few days in a desiccator over calcium chloride smelled strongly of oxides of nitrogen.

Aqueous solutions of the N-alkyl,N′-nitrosoguanidines react with primary and secondary amines, as if they were solutions of the alkylcyanamides, to produce di-(or tri-) alkyl-guanidines in which the substituent groups are attached to two different nitrogen atoms. The dearrangement and subsequent reaction are evidently as follows.

$$\begin{aligned} &\mathrm{RHN{-}C(NH){-}NH{-}NO} \rightleftarrows \\ &\qquad (\mathrm{RNCNH} \rightleftarrows \mathrm{RHN{-}CN}) + \mathrm{NH_2{-}NO} \\ &\mathrm{RHN{-}CN} + \mathrm{R'{-}NH_2} \longrightarrow \mathrm{RHN{-}C(NH){-}NHR'} \end{aligned}$$

When the amine in excess is added at laboratory temperature to an aqueous solution of a N-alkyl, N′-nitrosoguanidine, nitrogen commences to come off at once, and the liquid warms up slightly and on standing gradually fades to colorless. The addition of ammonium picrate solution causes the precipitation of the picrate of the N,N′-di-(or tri-) alkylguanidine. By this means we have prepared, in the indicated yields, and isolated and characterized by means of their picrates, the sub-

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[6] In a later paper of this series we expect to discuss the mechanism and the conditions for the formation of nitrous acid from nitrosoguanidine.

[7] Sabetta, Himmelfarb and Smith, THIS JOURNAL, **57**, 2478 (1935).

[8] Thiele, *Ann.*, **273**, 133 (1893).

stituted guanidines which are listed in Table I. The yields are calculated on the basis of the alkylnitroguanidine which was reduced for the preparation of the alkylnitrosoguanidine which was used in the reaction.

TABLE I

PICRATES OF N,N'-DI- AND TRIALKYLGUANIDINES

Guanidine picrates	Yield, %	M. p., °C.	Nitrogen, % Found	Nitrogen, % Calcd.
N,N'-Dimethyl-[9]	17.4	176.5–177.0	...	...
N-Methyl,N'-ethyl-	11.1	170.0–171.0	25.63, 25.54	25.45
N-Methyl,N'-*n*-butyl-	22.4	139.5–141.0	23.50, 23.62	23.43
N-Methyl,N'-piperido-	9.8	193.0	22.92, 22.98	22.70
N-Methyl,N'-benzyl-	9.3	196.0–197.0	20.90, 21.08	21.42
N-Ethyl,N'-*n*-butyl-	17.7	120.0	22.18, 22.40	22.58
N-*n*-Butyl,N'-piperido-	11.7	239.0–240.0	20.56, 20.82	20.38
N-*n*-Butyl,N'-benzyl-	16.6	171.0–172.0	19.35, 18.99	19.35
N,N,N'-Trimethyl-[10]	11.8	214.0	...	...
N,N-Dimethyl,N'-*n*-butyl	8.1	118.0	23.10, 22.91	22.58

Experiments

Preparation of Nitrosoguanidine.—In a typical experiment 21 g. of nitroguanidine, 11 g. of ammonium chloride, 18 g. of zinc dust (40% excess) and 250 cc. of water were stirred mechanically in an 800-cc. beaker while external cooling was applied to prevent the temperature from rising above 20–25°. After about two and one-half hours the gray color of the zinc had disappeared, the mixture was yellow, and on settling showed no crystals of nitroguanidine. The mixture was then cooled to 0° or below by surrounding the beaker with a mixture of ice and salt; it was filtered and the filtrate discarded. The yellow residue, consisting of nitrosoguanidine mixed with zinc oxide or hydroxide and basic zinc chloride, was extracted with four successive portions of 250 cc. each of water at 65°. The combined extracts, allowed to stand overnight at 0°, deposited nitrosoguanidine which was collected, rinsed with water, and dried at 40°, a yellow crystalline powder which exploded in a melting point tube at 165° as reported by Thiele; yield 8.0–9.2 g., 45–52%. Analysis of silver salt: found 55.65, 55.80%; calcd. for CH_3ON_4Ag, 55.45%.

Nitrosoguanidine and Ammonia.—A mixture of 125 cc. of strong ammonia water and 4 g. of nitrosoguanidine was evaporated to dryness on the steam-bath during four hours. The residue consisted of about 0.2 g. of insoluble white amorphous material, melamine, etc., and of colorless crystals of guanidine carbonate which, dissolved in water and treated with ammonium picrate, yielded 4.7 g. of guanidine picrate, 32.5% of the theoretical; in another experiment 34.2%.

Three grams of nitrosoguanidine was treated with fourteen successive portions of 50 cc. of strong ammonia, and allowed each time to evaporate spontaneously to dryness at room temperature. After the fourteenth evaporation the yellow color of the nitrosoguanidine had disappeared entirely. The residue yielded 0.6 g. of ammeline, insoluble in hot water but soluble in sodium carbonate, and 23.3% of the theoretical amount of guanidine determined as picrate.

Nitrosoguanidine and Ammonium Carbonate.—Ten grams of nitrosoguanidine, 50 g. of ammonium carbonate and 250 cc. of water were warmed together at 70° under reflux. Vigorous gas evolution occurred at first but had subsided after two hours, at the end of which time all of the materials had gone into solution. The mixture was refluxed for three hours to complete the reaction, then evaporated to dryness on the steam-bath, and yielded 7.3 g. of dry residue free from ammonium carbonate. The water-soluble portion of this material gave 7 g. of guanidine carbonate (80%) insoluble in alcohol, and a trace of urea, soluble in alcohol, while the portion insoluble in water gave 0.1 g. of melamine (2%), soluble in hot water, and 0.3 g. of ammelide (6%), soluble in potassium hydroxide solution and precipitated by acetic acid.

Alkylguanidines.—Our first experiments on the reaction of aliphatic amines with nitrosoguanidine were carried out at 60–70°, but, as the reaction proceeded vigorously at room temperature, the later experiments were conducted by suspending the nitrosoguanidine in water, adding the amine—using an excess of the more volatile amines—and allowing the mixture to stand in an unstoppered flask. After one or two days, the yellow color had disappeared and gas evolution had ceased. The slightly turbid liquid was filtered and precipitated with ammonium picrate. The picrates were recrystallized from alcohol or alcohol–water mixtures to constancy of melting point. We have operated in this way because we have wished to determine the yields of the alkylguanidines, and have made no effort at this time to procure these compounds in the more readily usable forms of their sulfates and carbonates. The yields of picrates were as follows: methylguanidine 21.2%, ethyl- 16.9%, *n*-butyl- 35.9%, *n*-heptyl- 35.4%, N,N-dimethyl- 21.4%, benzyl- 16.3%, and piperidoguanidine 37.2%. All of these picrates have been described by Davis and Elderfield[9] except ethylguanidine picrate, which is new.

Ethylguanidine picrate, yellow crystals from alcohol, melting sharply at 175°. Analysis for nitrogen: found 26.45, 26.55%; calcd. for $C_9H_{12}O_7N_6$, 26.58%.

In the experiment with benzylamine, 5 g. of nitrosoguanidine, 7 g. of benzylamine and 50 cc. of water were allowed to stand at room temperature for two days. The crystalline material which had separated was recrystallized from hot water and identified as *sym*-dibenzylurea by mixed melting point with a known sample. The solution on precipitation with ammonium picrate yielded 3.5 g. (16.3%) of benzylguanidine picrate.

N-Methyl,N'-nitrosoguanidine.—A mixture of 34.5 g. of N-methyl,N'-nitroguanidine, 28 g. of zinc dust, 16 g. of ammonium chloride and 150 cc. of 50% aqueous alcohol was stirred mechanically while the temperature was kept between 10 and 15° by the addition from time to time of chipped ice. After a few minutes the color of the mixture had changed from gray to green, and the odor of ammonia had become apparent. After five hours the color of the solution was deep red; the zinc had disappeared and a white solid was present in its place. The material was filtered, and the filtrate evaporated in a current of air yielded yellow needle crystals of crude methylnitrosoguanidine, 6.5 g. (21.8%). The filtrate from these crystals on further evaporation gave only a red sirupy mass.

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(9) Davis and Elderfield, THIS JOURNAL, **54**, 1502 (1932), reported m. p. 178°.

(10) Schenck and Kirchhoff, *Z. physiol. Chem.*, **153**, 150 (1926), reported m. p. 216°.

The methylnitrosoguanidine, recrystallized from alcohol at 50°, melted with decomposition at 95°. Analysis for nitrogen: found 54.19, 53.90%; calcd. for $C_2H_6ON_4$, 54.90%.

The reduction of methylnitroguanidine with zinc dust and aqueous ammonium hydroxide was carried out in the same way as with zinc dust and ammonium chloride except that it was slower, ten hours being required for its completion. The red filtrate from the reduction mixture deposited white flocculent material as long as it had any odor of ammonia. After all of the ammonia had disappeared, the liquid was filtered again, and the filtrate, now apparently free from zinc, on evaporation gave crystals of methylnitrosoguanidine or was used directly for the preparation of the silver salt which came down as a voluminous white precipitate when silver nitrate was added; yield of silver salt 39.6%. Analysis of silver salt of methylnitrosoguanidine: found Ag 51.74, 51.90%; calcd. for $C_2H_5ON_4$, Ag 51.19%.

N-*n*-Butyl-, and N-benzyl,N′-nitrosoguanidine, prepared by reduction of the nitro compounds with zinc dust and aqueous ammonium hydroxide, were procured as orange-red oils, rather unstable and very readily soluble in water. The zinc-free filtrates from the reduction mixtures were used directly for the preparation of the silver salts, yields, respectively, 30.9 and 32.9%. Analysis of silver salt of butylnitrosoguanidine: found Ag 42.76, 42.91%; calcd. for $C_5H_{11}ON_4$, Ag 42.96%. Analysis of silver salt of benzylnitrosoguanidine: found Ag 36.21, 36.42%; calcd. for $C_8H_{10}ON_4$, Ag 36.28%.

N,N′-Di- and trialkylguanidines, isolated as the picrates, were prepared by the interaction of the corresponding amines with the zinc-free red filtrates from the reduction of methyl-, *n*-butyl-, and benzylnitroguanidines by means of zinc dust and aqueous ammonium hydroxide. The procedure was the same as has been described for the reaction of nitrosoguanidine with aliphatic amines. N-Methyl,N′-*n*-butylguanidine prepared from the reaction of *n*-butylamine with methylnitrosoguanidine, yield 13.6%, was found, by a mixed melting point determination on the picrates, to be identical with that prepared from the reaction of methylamine with *n*-butylnitrosoguanidine, yield 22.4%.

Summary

Nitrosoguanidine reacts in aqueous solution with ammonia to produce guanidine, and with primary and secondary aliphatic amines to produce the corresponding alkylguanidines.

Nitrosoguanidine does not react with aniline or methylaniline in boiling aqueous solution but yields dicyandiamide quantitatively.

N-Methyl,N-*n*-butyl, and N-benzyl,N′-nitrosoguanidines have been prepared, the methyl compound as a crystalline solid, the others as orange-red oils.

N-Alkyl,N′-nitrosoguanidines react with primary and secondary aliphatic amines to produce di-(or tri-) alkylguanidines in which the substituent groups are attached to two different nitrogen atoms. Seven new N,N′-dialkylguanidines and one new N,N,N′-trialkylguanidine have been prepared by this reaction.

Nitrosoguanidine and the N-alkyl,N′-nitrosoguanidines dearrange in aqueous solution to produce cyanamide and the alkylcyanamides, respectively. The other product of the dearrangement is evidently nitrosoamide which accounts for the nitrogen which is evolved in these experiments.

CAMBRIDGE, MASS. RECEIVED AUGUST 24, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGY OF PRINCETON UNIVERSITY]

Chemical Studies on Bioluminescence. IV. Salt Effects on the Total Light Emitted by a Chemiluminescent Reaction[1]

BY RUBERT S. ANDERSON

An aqueous solution containing cypridina luciferin and the enzyme, cypridina luciferase, materials extracted from a small crustacean, emits light in the presence of oxygen.[2] The total light emitted during the reaction of a given volume of luciferin solution can be changed by various means,[3] and particularly it can be increased by the addition of sodium chloride to the reaction mixture. Other chemiluminescent reactions have not been studied in this way but salt effects on fluorescence are well known.[4] Since the radiation from fluorescent solutions is in general decreased by salts, if they have an effect at all, a more detailed study of the effects of sodium chloride and a series of other salts on the luminescent reaction of luciferin and luciferase has been made.

Experimental Procedure

The procedure and solutions have been described recently.[5] In addition the luciferase solutions were dia-

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(1) These results were presented at the Chapel Hill meeting of the American Chemical Society, April, 1937.

(2) E. Newton Harvey, "Nature of Animal Light," 1920, p. 103.

(3) Rubert S. Anderson, *J. Cell. Comp. Physiol.*, **3**, 45 (1933).

(4) W. West, R. H. Müller and E. Jette, *Proc. Roy. Soc.* (London), **A121**, 294, 299 (1928).

(5) Rubert S. Anderson, *J. Cell. Comp. Physiol.*, **8**, 261 (1936).