pared. A styryl derivative (IIIf) of khellin (IIIb) and khellin perchlorate are described. A reddish-violet color reaction of potassium hydroxide with khellin and certain other 2-methylchromones is discussed and the importance of the methyl group in position 2 for this reaction is stressed.

The physiological activities of norkhellin and its derivatives and of some simpler chromones and derivatives of khellinone is stated in figures beside the formulas, the activity of khellin being taken as 100. Some styryl derivatives described, show structural similarities to substances showing vitamin P activity.

Abbassia, Cairo, Egypt Received April 18, 1949

[Contribution from the Department of Chemistry, Queen's University]

The Chemistry of N-β-Substituted Ethyl-N′-nitroguanidines

By A. F. McKay[1] and J. E. Milks

The original purpose of this work was a study of the nitration products of N-β-hydroxyethyl-N′-nitroguanidine I. One of the products, N-β-nitroxyethyl-N′-nitroguanidine, was observed to undergo a change at elevated temperatures or on boiling with water. This observation led to a more extensive investigation of other β-substituted ethylnitroguanidines.

N-β-Hydroxyethyl-, N-β-chloroethyl-, N-β-bromoethyl-, N-β-methoxyethyl- and N-β-hydroxypropylnitroguanidine were prepared by a reaction previously described[2,3] by adding N-methyl-N-nitroso-N′-nitroguanidine to the corresponding amine in water or aqueous ethanol.

$$CH_3N(NO)—(CNH)—NHNO_2 + XCH(R)—CH_2NH_2 \longrightarrow XCH(R)—CH_2—NH—C(NH)—NHCO_2 + N_2\uparrow$$

I

Where X = OH, Cl, Br or OCH_3 and R = H or CH_3.

N-β-Hydroxyethyl-N′-nitroguanidine I on treatment with 2.3 mole equivalents of absolute nitric acid in acetic anhydride gave N-β-nitroxyethyl-N′-nitroguanidine IV. This latter compound melted at 107° resolidified at 108–109° and then melted with decomposition at 161. The crude product gave a negative secondary nitramine test[4,5] with dimethylaniline. On refluxing compound IV with water it became more soluble and it was necessary to concentrate the solution in order to obtain a product. The recovered product was more soluble in water and less soluble in organic solvents than the original N-β-nitroxyethyl-N′-nitroguanidine IV. This behavior suggested that a salt was formed which was confirmed by treating its aqueous solution with nitron[6] when an immediate quantitative precipitate of nitron nitrate formed. Compound VI gave a strong secondary nitramine test indicating that N-β-nitroxyethyl-N′-nitroguanidine had cyclized. This assumption was verified when 2,5-dinitro-2,5-diaza-1-cyclopentanone VII was obtained in 84% yield from compound VI on nitration with excess nitric acid in acetic anhydride. Compound VI may have either structure VI(a) or VI(b). In view of its failure to nitrosate structure VI(a) is preferred.

The linear compounds N-β-hydroxyethyl-N′-nitroguanidine I and N-β-nitroxyethyl-N′-nitroguanidine are easily nitrosated. The resulting yellow crystalline nitrosamines on treatment with aniline are converted into phenylnitroguanidine III.

On the careful addition of ammonium hydroxide to 1-nitro-2-amino-2-imidazoline hydrobromide the free base (m. p. 133.5°) is obtained.

Similar reactions were obtained with N-β-hydroxypropyl-N′-nitroguanidine which was converted into β-nitroxypropylnitroguanidine (m. p. 129–129.5°) by nitration. The latter compound was cyclized to 1-nitro-2-amino-5-methyl-2-imidazoline nitrate (m. p. 115–116°). Further proof of this cyclic structure was obtained by nitration to 2,5-dinitro-3-methyl-2,5-diaza-1-cyclopentanone.[7]

When N-β-hydroxyethyl-N′-nitroguanidine is nitrated with six mole equivalents of nitric acid in acetic anhydride a new compound VIII is obtained. This compound gives a strong secondary nitramine test[4] and analytical values in good agreement with N-β-nitroxyethyl-N-nitro-N′-nitroguanidine. This is the first reported[2] instance of the formation of a linear N,N′-dinitroguanidine derivative. Since this is a β-substituted ethylnitroguanidine it would also be expected to undergo cyclization with liberation of nitric acid on heating with water. This was found to be the case. This liberation of nitric acid is followed by hydrolysis and 1,2-dinitraminoethane IX (m. p. 176°) is isolated as the end-product. Compound VIII is also obtained from N-β-nitroxyethyl-N′-nitroguanidine IV on treatment with excess nitric acid in the presence of acetic anhydride.

(1) Present address: Defence Research Chemical Laboratories, Ottawa, Ontario.

(2) A. F. McKay and G. F. Wright, This Journal, **69**, 3028 (1947).

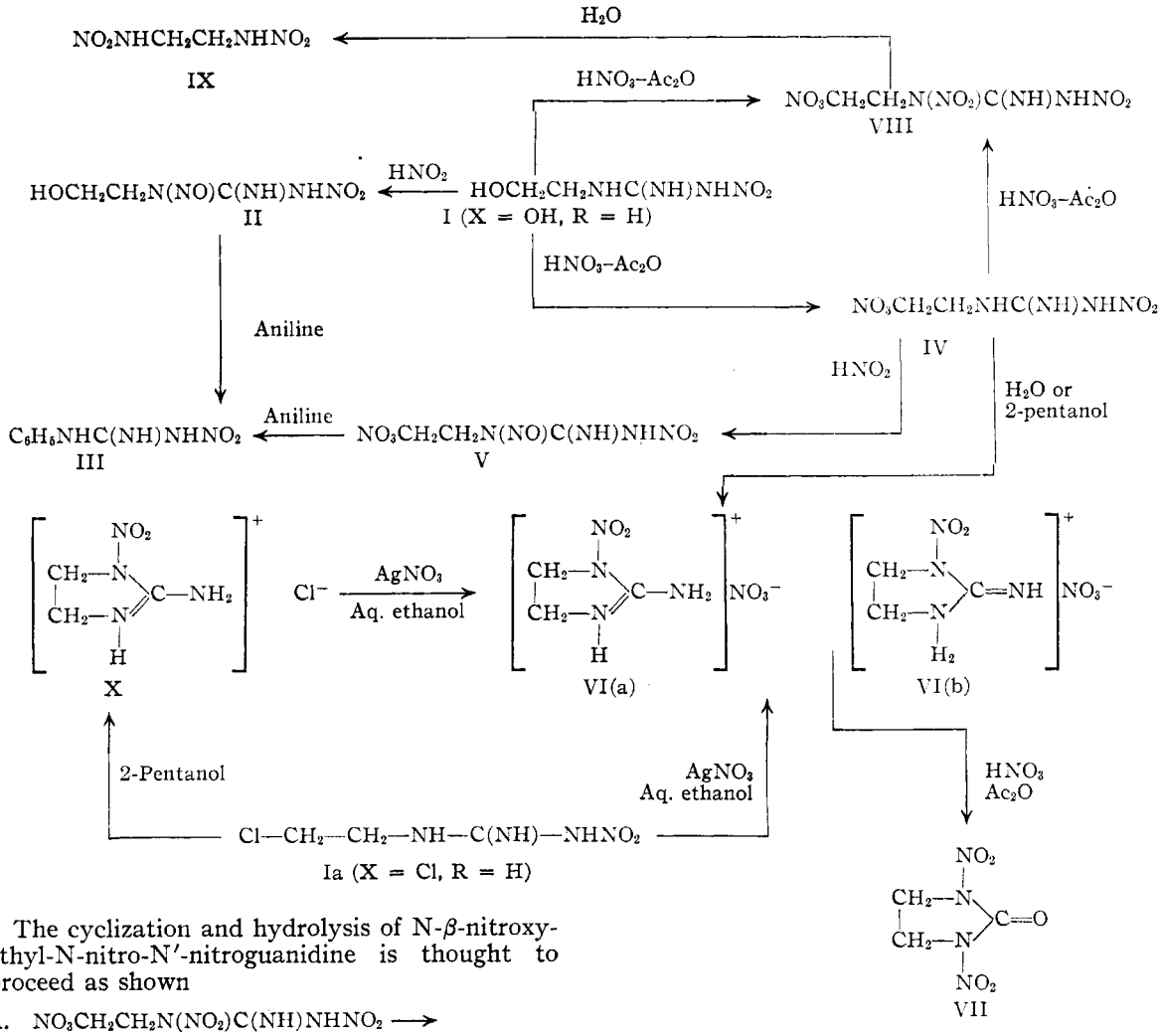
(3) A. F. McKay, *ibid.*, **71**, 1968 (1949).

(4) A. P. N. Franchimont, *Rev. trav. chim.*, **16**, 226 (1897).

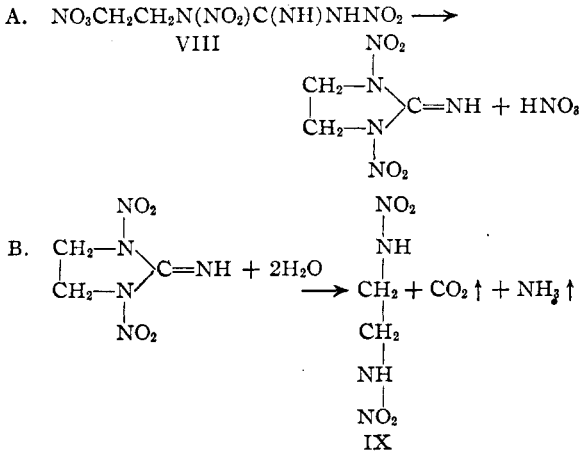
(5) On attempted purification of this compound some cyclization occurs and the product then gives a weak secondary nitramine test.

(6) E. C. Worden, "Nitrocellulose Industry," D. Van Nostrand Co., New York, N. Y., 1911, Vol. II, p. 768.

(7) A. F. McKay and D. F. Manchester, This Journal, **71**, 1970 (1949).



The cyclization and hydrolysis of N-β-nitroxyethyl-N-nitro-N'-nitroguanidine is thought to proceed as shown



The fact that free nitrate ion was identified as nitron nitrate at an intermediate stage in the hydrolysis of VIII supports reaction A. In order to establish the generality of this cyclization β-chloroethylnitroguanidine, β-bromoethylnitroguanidine and β-methoxyethylnitroguanidine were refluxed in water. β-Methoxyethylnitroguanidine was stable under these conditions but β-chloroethylnitroguanidine and β-bromoethylnitroguanidine both cyclized to 1-nitro-2-amino-2-imidazoline hydrochloride X and hydrobromide, respectively. It was found that better yields (83–100%) of the cyclized products could be obtained by adding the linear compounds to boiling 2-pentanol. Both β-chloroethylnitroguanidine Ia and 1-nitro-2-amino-2-imidazoline hydrochloride X give 1-nitro-2-amino-2-imidazoline nitrate VI on refluxing with an aqueous ethanol solution of silver nitrate.

β-Chloroethylnitroguanidine is nitrosated easily to N-β-chloroethyl-N-nitroso-N'-nitroguanidine. This compound on treatment with benzylamine gives benzylnitroguanidine.

Experimental[8]

N-β-**Hydroxyethyl-N'-nitroguanidine.**—Methylnitrosonitroguanidine (21.5 g., 0.146 mole) was added portionwise over a period of twenty-five minutes to a solution of 10.5 g. (0.172 mole) of ethanolamine in 20 cc. of water

(8) All melting points are uncorrected.

cooled to +5–10°. After all of the nitrosamine had been added, the reaction mixture was allowed to stand at room temperature for one hour. It was then cooled to 0° and the white product was filtered off and washed with cold water. The yield of crude product (m. p. 115° with decomposition) was 15.0 g. or 69.5%. Two crystallizations from 95% ethanol (6 cc./g.) gave 9.9 g. of pure β-hydroxyethylnitroguanidine melting at 118° with decomposition.

Anal. Calcd. for $C_3H_8N_4O_3$: C, 24.3; H, 5.40; N, 37.8. Found: C, 24.3; H, 5.45; N, 38.0.

β-Chloroethylamine Hydrochloride.—Sixty grams (0.985 mole) of ethanolamine was converted to the hydrochloride by adding an equivalent of concentrated hydrochloric acid. The water was removed *in vacuo* leaving the crystalline hydrochloride.

Ethanolamine hydrochloride was chlorinated by a modification of the procedure used by Ward.[9] To the total charge of ethanolamine hydrochloride 150 cc. of benzene and 156 g. (1.31 mole) of thionyl chloride were added. The reaction mixture was stirred at 60° for six hours, after which an additional 50 cc. of benzene and 26 g. (0.27 mole) of thionyl chloride were added. Then the temperature of the reaction mixture was raised to 75–85° for five hours. The product was filtered from the mixture and washed well with benzene, yield 100.4 g. or 88%. The crude product melted at 146–146.5°. One crystallization from 2-pentanol gave 91.7 g. of β-chloroethylamine hydrochloride melting at 147°. A melting point of 144° was previously reported.[9]

N-β-Chloroethyl-N′-nitroguanidine.—In this reaction the β-chloroethylamine was not isolated but the aqueous solution obtained from the addition of 88.5 cc. of 4 *N* sodium hydroxide solution to 41 g. (0.354 mole) of β-chloroethylamine hydrochloride was used. To this solution 26 g. (0.177 mole) of methylnitrosonitroguanidine was added under the conditions described for the preparation of β-hydroxyethylnitroguanidine. The crude product was obtained in 69% yield (20.3 g.) and melted at 116–117° with resolidification finally melting again at 185° with decomposition.

Anal. Calcd. for $C_3H_7ClN_4O_2$: C, 21.6; H, 4.20; N, 33.6. Found: C, 21.7; H, 4.22; N, 33.4.

N-β-Bromoethyl-N′-nitroguanidine.—This compound was prepared in 84% yield by a method analogous to the preparation of β-chloroethylnitroguanidine. β-Bromoethylnitroguanidine melted at 102–103°, resolidified and then melted with decomposition at 179–180°.

Anal. Calcd. for $C_3H_7BrN_4O_2$: C, 17.0; H, 3.32; N, 26.5. Found: C, 17.1; H, 3.20; N, 26.3.

N-β-Methoxyethyl-N′-nitroguanidine.—N-β-Methoxyethyl-N′-nitroguanidine was obtained under the conditions described for the preparation of β-hydroxyethylnitroguanidine. A 57% yield of β-methoxyethylnitroguanidine (m. p. 102–103°) was obtained. Two crystallizations from 95% ethanol (3.04 cc./g.) raised the melting point to 118.5–119.5°.

Anal. Calcd. for $C_4H_{10}N_4O_3$: C, 29.6; H, 6.17; N, 34.6. Found: C, 29.4; H, 6.31; N, 34.9.

N-β-Hydroxypropyl-N′-nitroguanidine.—N-β-hydroxypropyl-N′-nitroguanidine was prepared in 62.5% yield in a manner similar to that of β-hydroxyethylnitroguanidine. The melting point (103° with decomposition) was raised to 110–110.5° with decomposition by one crystallization from 95% ethanol.

Anal. Calcd. for $C_4H_{10}N_4O_3$: C, 29.6; H, 6.17; N, 34.6. Found: C, 29.6; H, 5.93; N, 34.8.

N-β-Nitroxyethyl-N′-nitroguanidine.—A suspension of 7.4 g. (0.050 mole) of β-hydroxyethylnitroguanidine in 20 cc. of acetic anhydride at 8° was mechanically stirred while 2.5 cc. (0.111 mole) of 99% nitric acid was added dropwise over a period of five minutes. The reaction mixture was then cooled to −5° and aged at this temperature for a further five minutes. The insoluble nitration product was removed on a fritted glass filter and washed with water. The product weighed 6.2 g. and melted at 109–110° resolidified and melted at 157–159° with decomposition. A second crop of 1.9 g. was obtained on dilution of the filtrate with ice-water and cooling to −20°. The combined yield was 84%. The product was crystallized twice from 95% ethanol (3.3 cc./g.) to give crystals melting at 92–93.5° resolidifying and remelting at 161° with decomposition.

Anal. Calcd. for $C_3H_7N_5O_5$: C, 18.6; H, 3.63; N, 36.2. Found: C, 18.8; H, 3.47; N, 36.0.

N-β-Nitroxypropyl-N′-nitroguanidine.—To a suspension of β-hydroxypropylnitroguanidine (11.2 g., 0.069 mole) in 50 cc. of acetic anhydride at 10° was added 3.44 cc. (0.152 mole) of 99.8% nitric acid over a period of five minutes. The mechanically stirred mixture was aged for a period of thirty minutes at −5°, after which the white insoluble product was recovered by filtration and washed with water. A crude yield of 11.7 g. (81.6%) was obtained. The melting point of 129–132° was changed to 129–129.5° by one crystallization from 95% ethanol (6.7 cc./g.).

Anal. Calcd. for $C_4H_9N_5O_5$: C, 23.2; H, 4.35; N, 33.8. Found: C, 23.1; H, 4.43; N, 34.0.

1-Nitro-2-amino-2-imidazoline Hydrochloride.—By Method A: Five grams (0.030 mole) of β-chloroethylnitroguanidine was refluxed with 10 cc. of distilled water. The clear solution was concentrated to 3 cc. and treated with 30 cc. of absolute alcohol. Ether was added until no further turbidity developed. The crystalline precipitate (4.3 g., 86% yield) was recovered by filtration. It melted at 180° with decomposition. This product was treated twice more, as above, to give 3.5 g. (70%) of product melting at 187.5–188° with decomposition.

Anal. Calcd. for $C_3H_7ClN_4O_2$: C, 21.6; H, 4.20; N, 33.6. Found: C, 21.5; H, 4.28; N, 33.4.

One gram (0.006 mole) of 1-nitro-2-amino-2-imidazoline hydrochloride was dissolved in 5 cc. of water and treated with a saturated picric acid solution. A crystalline picrate separated immediately, yield 2.0 g. (92.7%). The melting point of 188–189° was raised to 189.6° by one crystallization from 95% ethanol.

Anal. Calcd. for $C_9H_9N_7O_9$: C, 30.1; H, 2.50; N, 27.3. Found: C, 30.2; H, 2.70; N, 27.5.

Method B: When 1 g. (0.006 mole) of β-chloroethylnitroguanidine was refluxed with 10 cc. of 2-pentanol it dissolved but within a few minutes an insoluble product separated. The volume of 2-pentanol was decreased to 5 cc. by evaporation and the residue cooled to 0°. One gram (100% yield) of product was obtained melting at 188–189° alone and on admixture with an authentic sample of 1-nitro-2-amino-2-imidazoline hydrochloride.

1-Nitro-2-amino-2-imidazoline Hydrobromide.—β-Bromoethylnitroguanidine (4.2 g., 0.020 mole) when subjected to the procedure outlined in Method A gave 3.0 g. (71.5%) of 1-nitro-2-amino-2-imidazoline hydrobromide (m. p. 179.5° with decomposition). The use of Method B gave a quantitative yield melting at 179.5–180° with decomposition. This compound gave a strong secondary nitramine test.

Anal. Calcd. for $C_3H_7BrN_4O_2$: C,17.0; H, 3.32; Br, 37.9; N, 26.5. Found: C, 16.9; H, 3.29; Br, 37.2; N, 26.3.

1-Nitro-2-amino-2-imidazoline Nitrate: 1. From β-Nitroxyethylnitroguanidine.—β - Nitroxyethylnitroguanidine (6.7 g., 0.034 mole) when treated as in Method A gave 4.5 g. (67.1% yield) of 1-nitro-2-amino-2-imidazoline nitrate (m. p. 161° with decomposition).

Method B gave an 83% yield of the nitrate salt of 1-nitro-2-amino-2-imidazoline. The secondary nitramine test was very pronounced.

Anal. Calcd. for $C_3H_7N_5O_5$: C, 18.6; H, 3.63; N, 36.2. Found: C, 18.8; H, 3.36; N, 36.0.

A quantitative estimation of nitrate ion was carried out with "Nitron" using the procedure described by Worden.

(9) K. Ward, THIS JOURNAL, **57**, 914 (1935).

A quantitative yield (1.170 g. from 0.600 g. of 1-nitro-2-amino-2-imidazoline nitrate) of nitron nitrate was obtained.

2. From β-Chloroethylnitroguanidine.—β-Chloroethylnitroguanidine (10 g., 0.06 mole) was added to a solution of 10.4 g. (0.061 mole) of silver nitrate in 55 cc. of 58% ethanol. After refluxing for fifteen minutes, the hot mixture was filtered. The filtrate on cooling deposited 8.5 g. (72.4%) of white crystals which melted at 160° with decomposition. A mixed melting point determination with an authentic specimen of 1-nitro-2-amino-2-imidazoline nitrate (m. p. 161° with decomposition) was not depressed.

3. From 1-Nitro-2-amino-2-imidazoline Hydrochloride.—When 2.5 g. (0.015 mole) of 1-nitro-2-amino-2-imidazoline hydrochloride was treated under the conditions of procedure 2, 1.7 g. (58% yield) of white crystals was obtained. These crystals melted at 160° with decomposition alone and on admixture with a sample of 1-nitro-2-amino-2-imidazoline nitrate.

1-Nitro-2-amino-5-methyl-2-imidazoline Nitrate.—Four grams (0.019 mole) of β-nitroxypropylnitroguanidine was refluxed with 25 cc. of water. The aqueous solution was allowed to concentrate to *ca.* 10 cc. when the remainder of the water was removed *in vacuo*. The residue was treated with absolute alcohol and ether and filtered, yield 3.2 g. (80%). This product melted at 115–116° and the melting point was not changed by crystallizing from a mixture of benzene and absolute ethanol (2.5:1).

Anal. Calcd. for $C_4H_9N_5O_5$: C, 23.2; H, 4.35; N, 33.8. Found: C, 23.1; H, 4.43; N, 33.7.

2,5-Dinitro-2,5-diaza-1-cyclopentanone from 1-Nitro-2-amino-2-imidazoline Nitrate.—1-Nitro-2-amino-2-imidazoline nitrate was converted to 2,5-dinitro-2,5-diaza-1-cyclopentanone in 84% yield by the procedure described by McKay and Wright[10] for the preparation of dinitroethyleneurea. It was identified by a mixed melting point determination and hydrolysis to 1,2-dinitraminoethane (m. p. 176°).

2,5-Dinitro-3-methyl-2,5-diaza-1-cyclopentanone.—1-Nitro-2-amino-5-methyl-2-imidazoline nitrate was nitrated to give 2,5-dinitro-3-methyl-2,5-diaza-1-cyclopentanone (m. p. 99–100°) in 37% yield. The procedure followed has been described by McKay and Manchester[8] for the preparation of 2,5-dinitro-3-methyl-2,5-diaza-1-cyclopentanone, with the exception that the reaction temperature was 45–50° instead of 24°. The identity of the product was verified by a mixed melting point determination.

1-Nitro-2-amino-2-imidazoline.—To a solution of 1.8 g. (0.0085 mole) of 1-nitro-2-amino-2-imidazoline hydrobromide in 4 cc. of water, 4.5 cc. of concentrated ammonium hydroxide solution was added. After a few minutes, a white crystalline precipitate separated. This product was filtered off and washed with a few cc. of cold water, yield 0.8 g. (72%). The melting point of 131° was raised to 133.5° after two crystallizations from 95% ethanol (6.2 cc./g.).

Anal. Calcd. for $C_3H_6N_4O_2$: C, 27.7; H, 4.61; N, 43.0. Found: C, 27.6; H, 4.45; N, 43.1.

N-β-Nitroxyethyl-N-nitro-N'-nitroguanidine: 1. From β-Hydroxyethylnitroguanidine.—A nitration mixture of 2.75 cc. (0.113 mole) of 99.8% nitric acid in 5.80 cc. of acetic anhydride was prepared at 0° and maintained at this temperature for a period of five minutes while 2.80 g. (0.0189 mole) of β-hydroxyethylnitroguanidine was added. The temperature then rose spontaneously after which it was held at 40–47° for thirty minutes. During this time a considerable quantity of gas was evolved. Finally the reaction mixture was poured onto *ca.* 50 g. of ice. The white solid was filtered off and washed with water, yield 1.1 g. (24.4%). The crude product (m. p. 81–82°) was purified by dissolving in boiling methanol (6 cc./g.), adding water (2 cc./g.) and then allowing to cool. The pure product melted at 84.5–85.3°, yield 0.7 g. This compound gave a strong secondary nitramine color reaction.

Anal. Calcd. for $C_3H_6N_6O_7$: C, 15.1; H, 2.52; N, 35.3. Found: C, 15.1; H, 2.62; N, 35.5.

2. From β-Nitroxyethylnitroguanidine.—β-Nitroxyethylnitroguanidine (4.0 g., 0.020 mole) was added to a solution of 1.95 cc. (0.092 mole) of 99.8% nitric acid in 6.3 cc. of acetic anhydride at 0° over a period of five minutes. Then the mixture was heated in a water-bath at 50° for thirty-five minutes after which it was poured onto ice. The precipitate was filtered off and washed with water, yield 1.7 g. (34.8%). The purified product (1.5 g.) melted at 84.7–85.3° alone and admixed with an authentic sample.

1,2-Dinitraminoethane from N-β-Nitroxyethyl-N-nitroguanidine.—N - β - Nitroxyethyl - N - nitro - N' - nitroguanidine (1.4 g., 0.006 mole) was refluxed with 15 cc. of water. A clear solution resulted after two minutes refluxing. This solution was concentrated by evaporation to one-third its original volume, cooled to 0° and the crystals removed by filtration. This procedure was repeated twice to yield 0.5 g. (56.8%) of 1,2-dinitraminoethane (m. p. 174–175°). One crystallization from 95% ethanol gave crystals melting at 176° alone and on admixture with a known sample.

N-β-Hydroxyethyl-N-nitroso-N'-nitroguanidine.—Nine grams (0.061 mole) of β-hydroxyethylnitroguanidine was dissolved in 21 cc. of 70% nitric acid and the solution diluted with 45 cc. of water. The nitrosation was effected by the use of 13.5 g. (0.196 mole) of sodium nitrite as previously described[2] for the nitrosation of alkylnitroguanidines. A 37.2% yield of yellow crystalline nitrosamine was obtained which melted at 111.5° with decomposition. Three crystallizations from absolute methanol (4 cc./g.) did not change the melting point.

Anal. Calcd. for $C_3H_7N_5O_4$: C, 20.3; H, 3.95; N, 39.5. Found: C, 20.1; H, 3.80; N, 39.9.

This compound was unstable and decomposed on standing in the dark over a period of seven days.

Two grams of N-β-hydroxyethyl-N-nitroso-N'-nitroguanidine on treatment with aniline[2,3] gave a 33% yield of phenylnitroguanidine (m. p. 151–152°). This sample gave no depression in melting point when mixed with a known sample of phenylnitroguanidine (m. p. 152–153°).

N-β-Nitroxyethyl-N-nitroso-N'-nitroguanidine.—N-β-Nitroxyethyl-N'-nitroguanidine when nitrosated as above gave a 54% yield of N-β-nitroxyethyl-N-nitroso-N'-nitroguanidine (m. p. 105° with decomposition). One crystallization from 95% ethanol (4.85 cc./g.) gave a yellow crystalline product melting at 112.5° with decomposition.

Anal. Calcd. for $C_3H_6N_6O_6$: C, 16.2; H, 2.70; N, 37.8. Found: C, 16.5; H, 2.76; N, 37.4.

N-β-Nitroxyethyl-N-nitroso-N'-nitroguanidine on treatment with aniline[2,3] gave a 37% yield of phenylnitroguanidine (m. p. 152–153°) identified by a mixed melting point determination.

N-β-Chloroethyl-N-nitroso-N'-nitroguanidine.—Nitrosation of β-chloroethylnitroguanidine as above gave N-β-chloroethyl-N-nitroso-N'-nitroguanidine (m. p. 96° with decomposition) in 69% yield. One crystallization from absolute methanol (5.3 cc./g.) raised the melting point to 114.5° with decomposition.

Anal. Calcd. for $C_3H_6ClN_5O_3$: C, 18.4; H, 3.07; N, 35.8. Found: C, 18.6; H, 3.14; N, 35.4.

Phenylnitroguanidine (m. p. 152–153°) and benzylnitroguanidine (m. p. 183°)[2,3] were prepared from N-β-chloroethyl-N-nitroso-N'-nitroguanidine in 38 and 74% yield, respectively.

Acknowledgment.—The authors wish to thank the Defence Research Board of Canada for a grant-in-aid in support of this work.

Summary

Some members of the N-β-substituted ethyl-N'-nitroguanidine series have been shown to

(10) A. F. McKay and G. F. Wright, THIS JOURNAL, **70**, 3990 (1948).

undergo a new type of cyclization reaction. The reactions of both the linear and cyclic compounds are described.

A linear N,N′-dinitroguanidine (N-β-nitroxyethyl-N-nitro-N′-nitroguanidine) has been prepared for the first time. It cyclizes on boiling with water splitting off nitric acid. The cyclized product then hydrolyzes to give 1,2-dinitraminoethane.

KINGSTON, ONTARIO RECEIVED AUGUST 26, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY, AND THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, TUFTS COLLEGE MEDICAL SCHOOL]

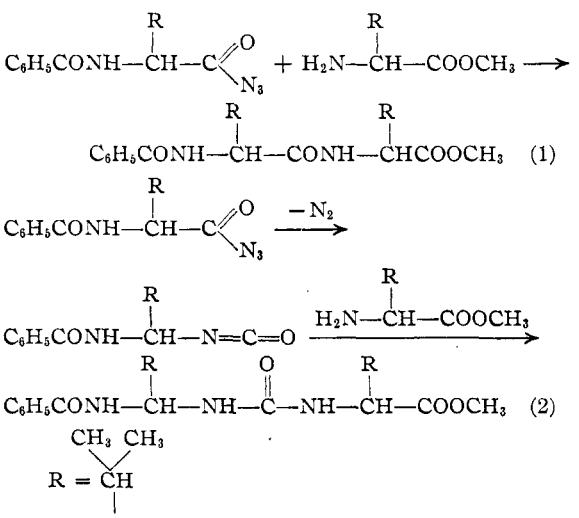
The Isomeric Dipeptides of Valine Including a Correction[1]

BY J. W. HINMAN, E. LOUIS CARON AND HALVOR N. CHRISTENSEN

A dipeptide of astonishing stability was reported to survive the acid hydrolysis of gramicidin and to be present in not one but at least two isomeric forms, namely, D-valyl-D-valine and L-valyl-L-valine.[2,3] The evidence for this view was as follows: low or absent optical activity of the benzoyl derivative; isolation of DL-valine after acid hydrolysis, recovery of half the valine released during hydrolysis as L-valine by microbial assay; comparison of the isolated product with two synthetic mixtures supposed to represent enantiomorphic pairs. Racemization was subsequently observed by the original investigator in the synthetic reaction employed. The product of the reaction of benzoyl-D-valyl chloride and D-valine ethyl ester upon hydrolysis yielded 27 and 30% of the valine as microbiologically available L-valine.[4] Recent studies[5] have pointed out the optical instability of acylamino acid halides. Therefore the problem has been reinvestigated jointly with the conclusion that the dipeptide from gramicidin does indeed consist of a mixture of two enantiomorphic forms, these being D-valyl-L-valine and L-valyl-D-valine, however, rather than the pair originally supposed.

Synthesis

Positive identification of the valylvaline from gramicidin required the synthesis of the isomeric dipeptides of valine by a method which would not permit racemization at any point. Since the benzoyl derivative of the dipeptide from gramicidin was available, the condensation of benzoylvalylazide and valine methyl ether was investigated. However, under conditions normally employed for such a condensation (reaction 1) very little of the desired product was obtained, the major product being the ureide as indicated in reaction 2. Even under the mild conditions employed, the acid azide underwent the Curtius



(1) An abstract of this paper was presented before the Division of Biological Chemistry at the 116th National meeting of the American Chemical Society in Atlantic City, September, 1949.

(2) Abbreviated, DvDv and LvLv.

(3) (a) H. N. Christensen, *J. Biol. Chem.*, **151**, 319 (1943); (b) **154**, 427 (1944).

(4) D. M. Hegsted, *ibid.*, **152**, 193 (1944).

(5) H. E. Carter and J. W. Hinman, *ibid.*, **178**, 403 (1949).

rearrangement before reacting with valine methyl ester. This reaction has been encountered with several other benzamido acid azides and the factors influencing this reaction are now being studied.

The *p*-toluenesulfonyl (tosyl) derivatives of the four optical isomers of valylvaline were prepared by condensing optically active tosylvalyl chloride with optically active valine methyl ester. Preparation of the free dipeptides by removal of the tosyl group through the action of sodium in liquid ammonia did not proceed smoothly; therefore, carbobenzoxy-DL-valyl chloride was condensed in separate reactions with D-valine methyl ester and with L-valine methyl ester to give in each case a mixture of two diastereoisomers of carbobenzoxyvalylvaline methyl ester.[6] The diastereoisomers were readily separated by fractional crystallization. The carbobenzoxy group was removed from one of the pure isomers by catalytic hydrogenation, the valylvaline ester tosylated, and the product identified by com-

(6) It is interesting to note that Polglase and Smith (THIS JOURNAL, **71**, 3081 (1949)) have recently prepared carbobenzoxy-L-leucyl-D-alanine methyl ester and carbobenzoxy-L-leucyl-L-alanine methyl ester from carbobenzoxy-L-leucine and DL-alanine methyl ester. This practice of preparing and separating diastereoisomers of dipeptides, as they point out, has not been widely used, but is particularly useful in some cases.