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**NOTE ON THE CONVERSION OF THE AMINO GROUP OF AMINO ACIDS INTO THE NITROGUANIDINO GROUP**

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A study has been made of 2-methyl-1-nitroisourea as a reagent for the conversion of amino groups of amino acids into guanidino groups. This reagent has proved its usefulness in modifying the  $\omega$ -amino groups of diaminocarboxylic acids in particular. The reaction of 2-methyl-1-nitroisourea with glycine, L-ornithine and D- and L-lysine to yield nitroguanidinoacetic acid, L-nitroarginine and D- and L-nitro-homoarginine, respectively, is described.

*Saroff* and *Evans*<sup>1</sup> described the conversion of the amino group of some amino acids into the nitroguanidino group with the use of 2-methyl-1-nitroisothiourea<sup>2</sup>. Attempts to apply this reagent to  $\alpha,\omega$ -diamino acids were unsuccessful. We attributed the cause of this failure to a reaction of the copper, used for masking the  $\alpha$ -amino group by complex formation<sup>3</sup>, with the sulphur-containing compounds present\*. We therefore synthesized 2-methyl-1-nitroisourea [Me—O—C(NH<sub>2</sub>)=N—NO<sub>2</sub>]. This compound could be obtained from O-methylisourea hydrosulphate by nitration. The latter substance was prepared by methylation of urea with dimethyl sulphate essentially according to the method of *Fearing* and *Fox*<sup>4,5</sup>; (see Experimental). Nitration of the hydrosulphate yielded a product, which, at room temperature, decomposed in the nitrating medium. It is therefore important that the nitration mixture be poured on to such an amount of crushed ice that the temperature remains close to -15°. The desired product was obtained in a yield of 50%. The

\* A greyish yellow precipitate formed. *Kurtz* reported<sup>3</sup> the formation of a similar precipitate when for the guanidation of  $\alpha,\omega$ -diamino acids he used  $\alpha$ -Cu complexes, chloro-2-methylisothiourea and bromo-2-ethylisothiourea.

<sup>1</sup> *H. A. Saroff* and *R. L. Evans*, *Biochim. Biophys. Acta* **36**, 511 (1959).

<sup>2</sup> *L. Fishbein* and *J. A. Callaghan*, *J. Am. Chem. Soc.* **76**, 1877 (1954).

<sup>3</sup> *A. C. Kurtz*, *J. Biol. Chem.* **180**, 1253 (1949).

<sup>4</sup> *R. B. Fearing* and *S. W. Fox*, *J. Am. Chem. Soc.* **76**, 4384 (1954).

<sup>5</sup> *J. W. Janus*, *J. Chem. Soc.* **1955**, 3551.

dry methylnitrosoourea is a stable crystalline substance, m.p. 110°. Using this reagent the lysine and ornithine derivatives were obtained in yields of over 50% (D- and L-nitrohomoarginine proved to crystallize at a considerably slower rate from water-alcohol than nitroarginine). To obtain a good yield (74%) of nitroguanidinoacetic acid the pH of the reaction medium must be adjusted to 11 before adding 2-methyl-1-nitrosoourea\*.

### Experimental\*\*

The melting points were taken on a "Büchi" m.p. apparatus and are uncorrected.

#### 2-Methyl-1-nitrosoourea

a) O-Methylisourea hydrosulphate is prepared by a method involving a slight modification of the *Fearing* and *Fox* procedure according to which the reaction is allowed to proceed at a lower temperature and for a longer time<sup>5</sup>. A mixture of 150 g of urea and 315 g of dimethyl sulphate is stirred at 40-50° for 6 hours. On crystallization, 120 g (28%) of the product, m.p. 116-119°, are collected. (Litt.<sup>4</sup> 119°).

#### b) Nitration.

The hydrosulphate is dissolved at 0° in a mixture of 120 ml of fuming nitric acid (sp.gr. 1.5) and 280 ml of commercial sulphuric acid containing 98% H<sub>2</sub>SO<sub>4</sub>; stirring at 0-5° is continued for 2 hours. The mixture is poured with stirring on to 1.5 kg of crushed ice, the temperature dropping to ~-15°. After stirring another 10 mins. the ice is removed by filtration through a Buchner funnel without filter paper, the crystalline material is immediately collected on a glass filter and sucked as dry as possible. The crude product is dissolved in 400 ml of ethyl acetate and solid potassium carbonate is added portion-wise until the evolution of carbon dioxide on further additions ceases. The solution is dried over anhydrous magnesium sulphate and concentrated to half its volume. An equal volume of light petroleum (b.p. 60-80°) is added to the boiling solution; on cooling to 0°, 41 g (50%) of a white crystalline solid (m.p. 108-110°) is obtained. Crystallization from ethyl acetate-petroleum ether gave a product melting at 110°.

Found : C 20.3 ; H 4.3 ; N 35.3

Calc. for C<sub>2</sub>H<sub>5</sub>N<sub>3</sub>O<sub>3</sub> (118.99): ,, 20.17; ,, 4.20; ,, 35.29.

#### Nitroguanidinoacetic acid

A solution is prepared from 3 g of glycine and 30 ml of H<sub>2</sub>O. The pH is adjusted to 11 with 20 ml of 2 N NaOH and the solution is cooled to 0°. Powdered 2-methyl-1-nitrosoourea (4.76 g) is added in small portions with stirring, during which the temperature is not allowed to rise above 2°. When the pH has dropped to a constant value of 9.5-10, the reaction mixture is acidified with 20 ml of 3 N HCl to pH 1-2. The white precipitate formed, is crystallized from

\* The observation was made that on adding NaOH to a cooled solution of glycine and the reagent, at pH 9.5, gas evolution occurred and no product could be isolated.

\*\* The analyses were carried out by Mr. N. W. Louwrier.

water; yield 4.8 g. The substance decomposes at 179°. *Saroff and Evans*<sup>1</sup> report an indefinite melting point at about 180° with decomp. The equivalent weight was determined titrimetrically to be  $162 \pm 2$  (calc.: 162).

Found : C 22.3 ; H 3.8 ; N 34.4  
 Calc. for  $C_2H_6N_4O_4$  (161.98): ,, 22.22; ,, 3.70; ,, 34.57.

#### L-Nitrohomoarginine

L-lysinedihydrochloride (35 g) is dissolved in 150 ml of water. To this solution 10 g of copper carbonate are added and stirring is continued until a clear solution is obtained. The pH of the solution is adjusted to 11 by addition of 40% NaOH. The mixture is then cooled down to 0° with stirring and a total of 19 g of the powdered 2-methyl-1-nitroisourea is added at this temperature in small portions, the pH dropping to 9.5-10. When the pH remains constant for 10 minutes, the solution is acidified with concentrated HCl to pH = 2. The resulting clear solution is neutralized with 40% NaOH at which point the copper complex of nitrohomoarginine precipitates. After standing overnight at 0°, the blue crystalline complex is filtered, washed with methanol and ether and dried. The yield amounts to 37 g. The complex is dissolved in as little dilute hydrochloric acid as possible, the volume is made up to 200 ml with water and hydrogen sulphide is passed through until all of the copper has been precipitated. The latter is removed by filtration with the aid of Supercel and the volume is reduced to 100 ml by distillation under reduced pressure. The pH is adjusted to 8 with 25% ammonia and finally brought to 6 with glacial acetic acid<sup>6</sup>. Alcohol (800 ml) is added and the solution is allowed to stand at 0° for 3 days; 20 g (54%) of L-nitro-homoarginine are isolated. The white substance melts at 227-230° (decomp.),  $[\alpha]_D^{20} = +19.5^\circ$  (c = 2 in 2 N HCl). Crystallization from water/alcohol yields a product of m.p. = 230° (decomp.),  $[\alpha]_D^{20} = +20.7^\circ \pm 0.3^\circ$ . By means of formalin titration the equivalent weight is determined to be  $235 \pm 2$  (calc.: 233).

Found : C 35.8 ; H 6.8 ; N 30.1  
 Calc. for  $C_7H_{15}N_5O_4$  (232.98): ,, 36.06; ,, 6.44; ,, 30.05.

#### D-Nitrohomoarginine

From 11 g of D-lysine dihydrochloride 6.3 g (55.5%) of D-nitrohomoarginine are obtained, m.p. = 217-220°,  $[\alpha]_D^{20} = -18.8^\circ$  (c = 2 in 2 N HCl). Crystallization from water/alcohol gave D-nitrohomoarginine, m.p. = 231°,  $[\alpha]_D^{20} = -20.5^\circ \pm 0.3^\circ$ .

Found : C 35.8 ; H 6.8 ; N 30.1  
 Calc. for  $C_7H_{15}N_5O_4$  (232.98): ,, 36.06; ,, 6.44; ,, 30.05.

#### L-Nitroarginine

The starting materials are 4.1 g of L-ornithinedihydrochloride in 20 ml of water, 1.23 g of copper carbonate and 2.4 g of 2-methyl-1-nitroisourea. The method used is analogous to the one described for L-nitrohomoarginine. However, no alcohol is added to the neutralized solution as crystallization of nitroarginine occurs instantaneously on cooling. Yield: 2.3 g (52%), m.p. = 240-247° (decomp.). On crystallization from water/alcohol the melting point is 251°

<sup>6</sup> *K. Hofmann, W. D. Peckham and A. Rheiner, J. Am. Chem. Soc. 78, 238 (1956).*

(decomp.) (Litt. <sup>6</sup> 251-252°) <sup>6</sup>,  $[\alpha]_D^{20} = + 24.5^\circ \pm 0.3^\circ$  (c = 2 in 2 N HCl),  
 $[\alpha]_D^{25} = + 24.3^\circ$  (c = 4 in 2 N HCl) <sup>6</sup>.

Found : C 32.8 ; H 6.2 ; N 32.2  
Calc. for C<sub>6</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub> (218.98): ,, 32.98; ,, 5.94; ,, 31.98.

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