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# α-Diketone Rearrangement. The Reaction of 1,2-Cyclohexanedione with Arginine and with Guanidine

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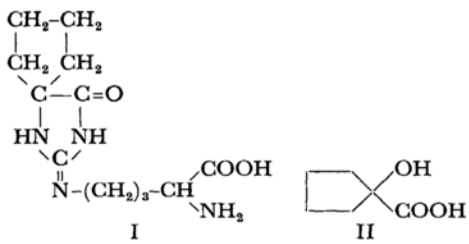
A novel rearrangement reaction of cyclohexanedione, involving the loss of hydrogen in the product, is described. Cyclohexanedione and guanidine react in an alkaline solution to produce 2-amino-4-oxo-1,3-diazaspiro[4.4]non-1-ene (VIII) and 1-hydroxy-3-amino-5-oxo-2,4-diazabicyclo-[4.3.0]non-2-ene (IX), the latter being an oxidized product. Similarly, cyclohexanedione reacts with arginine, producing, among other products, $N^5$-(1-hydroxy-5-oxo-2,4-diazabicyclo[4.3.0]non-2-ylidene)-ornithine (III).

Toi *et al.*[1] have reported that arginine reacts with cyclohexanedione (CHD) in basic solutions to produce $N^5$-(4-oxo-1,3-diazaspiro[4.4]non-2-ylidene)-L-ornithine (I). Though in strongly basic solutions the yield of I is almost quantitative, the presence of three minor products has also been shown by paper electrophoresis.[1] This reaction is unique in the sense that such rearrangements of aliphatic α-diketones similar to the benzilic-acid rearrangement have scarcely been known except that CHD is transformed to 1-hydroxycyclopentanecarboxylic acid[2] (II) in a concentrated solution of sodium hydroxide on heating.



I II

A paper chromatogram of the reaction mixtures of CHD and arginine is schematically shown in Fig. 1. The products are all isolated by cellulose column chromatography, and the ratios of the products are found to be markedly dependent on the concentrations of sodium hydroxide, as was suggested, by Toi *et al.*[1] If the reactions are carried out at pHs of less than 11, one unidentified product (corresponding to the spot 3 in Fig. 1) is found to be predominant. Though we at first incorrectly anticipated that it might be the first intermediate, through which I is produced, the structural elucidation of this compound has unexpectedly resulted in showing a novel reaction of CHD involving an intermolecular hydride shift. This report will be concerned with the structure of the product obtained from arginine and CHD at low concentrations of sodium hydroxide.

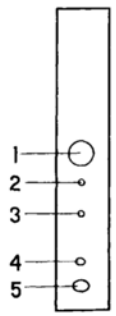



Fig. 1. The product distribution of a reaction mixture of arginine and cyclohexanedione in 0.2N NaOH: paper chromatogram developed with butanol, acetic acid, and water (4:1:2).

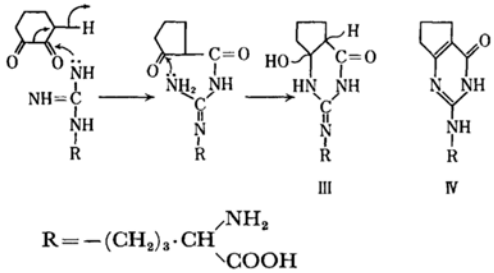
CHD and arginine were allowed to react in a 0.05N sodium hydroxide solution or at pH 11 at room temperature, and the products were isolated by cellulose column chromatography. The analytical data of the major product are consistent with the molecular formula of $C_{12}H_{20}N_4O_4$ after it has been heated at 140°C for 5 hr,[3] showing that CHD and arginine react with the loss of one mole of water and two atoms of hydrogen. A mechanistic consideration leads to two possible structures, III and IV (Scheme 1).

Though molecular-weight determination by the

1) K. Toi, F. Bynum, E. Norris and H. A. Itano, *J. Biol. Chem.*, **242**, 1036 (1967).

2) O. Wallach, *Ann.*, **437**, 174 (1924).

3) When the sample was dried over phosphorus pentoxide at room temperature, the analytical data were consistent with $C_{12}H_{20}N_4O_4 \cdot H_2O$. The water of crystallization was hardly expelled on heating at 100°C for a prolonged time over phosphorus pentoxide. When it was heated over 140°C, it gradually decomposed.

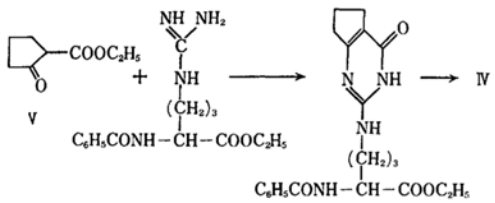


Scheme 1

parent peak seems rather to support the structure IV (Calcd for the trifluoroacetylated methyl ester of $C_{12}H_{18}N_4O_3$, 376.1358; measured, 376.1327), other physical and chemical data, to be discussed below, can more be reasonably explained by the structure III. IV is formally a dehydrated product of III, and the molecular ion of III may be rapidly dehydrated in a mass spectrometer, resulting in the loss of the parent peak.

Though the NMR spectrum is not very useful in elucidating the structure because of unanalyzable broad methylene signals, one methine proton signal at $\delta$ 4.10, a broad triplet, is inconsistent with a possible structure IV. The UV spectrum of the compound, $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 217 (17100), is almost the same as that of I, $\lambda_{max}^{H_2O}$ m$\mu$($\varepsilon$) 214 (18500), supporting the idea that both have the same chromophore, –NH–C(=N–)–NHCO. The compound IV has an aromaticity and is expected to have an absorption maximum at a longer wavelength.

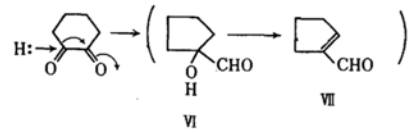
$N^5$-(5,6-Trimethylene-4-oxopyrimid-2-yl)ornithine (IV) was synthesized by the condensation of 2-ethoxycarbonylcyclopentanone (V) with benzoyl arginine ethyl ester, followed by acid hydrolysis (Scheme 2); as expected, IV is found to have a UV spectrum, $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 230 (8800), 292 (6100), similar to that of isocytosine, $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 222 (10000), 285 (5000). The mass spectra of III and IV (measured as the trifluoroacetylated methyl esters) are quite similar, strongly suggesting that the molecular ion of III is dehydrated with no observable parent peak to show a mass spectrum indistinguishable from that of IV.



Scheme 2

Though III shows a single spot on TLC, the possibility that it is a mixture of configurational isomers cannot be excluded.

After it has been established that an equimolar amount of CHD and arginine react, with the loss of two hydrogen atoms, to produce $N^5$-(1-hydroxy-5-oxo-2,4-diazabicyclo[4.3.0]non-2-ylidene)ornithine (III), the next problem is to determine the place where the hydrogen is accepted. As no evolution of gas is observed during the reaction, it seems reasonable to assume that CHD is reduced to 1-hydroxycyclopentanecarboxyaldehyde (VI) or 1-cyclopentene-1-carboxyaldehyde (VII), which might then suffer from further reactions (Scheme 3).



Scheme 3

Though the effort to isolate an aldehyde failed, possibly because of the susceptibility of aldehydes under these reaction conditions and because of the difficulty of separating an aldehyde from an excess of CHD, the positive reactions with Angeli's[4] and Tollens' reagent, after CHD and arginine have reacted in basic solutions, strongly suggest that two moles of CHD are needed for the formation of III; one reacts with arginine, and the other accepts the hydride anion. The following experimental findings seem to support this presumption: the spectrophotometric determination of the products on TLC shows that, when the reaction is carried out in a weakly basic solution, III is the only product, accompanied by an equimolar amount of unreacted arginine, and the use of an excess of CHD, an elevated temperature, and a prolonged reaction time have no influence on the ratio of the III to the arginine. We have still to answer the question of why half the arginine must remain unreacted. This peculiarity can be explained by presuming the following reaction path, which involves the formation of an aldehyde from CHD (Scheme 4):

2CHD + arginine → an aldehyde + III (1)

an aldehyde + arginine → a Schiff base of arginine (2)

Scheme 4

Generally, aldehydes are known to form Schiff bases more easily than ketones, and it is expected that an aldehyde, if it is involved in the reaction path on the formation of III (Eq. (1)), will at once combine with arginine to form a Schiff base (Eq. (2)), which is a species unable to react with CHD. Thus, the fact that only half the arginine is transformed to III in weakly basic solutions can be reasonably explained.

When the reaction is carried out in the presence of silver nitrate without altering the other reaction

4) A. Angeli, *Gazz. Chim. Ital.*, **34**, I, 50 (1904).

conditions, arginine is found to be almost completely consumed, being transformed to III.

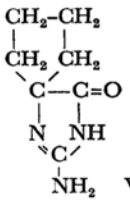
Two mechanism of the action of the silver cation are possible (Scheme 5): it might accept an electron from the hydride anion (Eq. (3)), or it might oxidize an aldehyde to prevent arginine from forming a Schiff base (Eq. (4)):

$$H^- + 2Ag^+ \rightarrow 2Ag + H^+ \quad (3)$$

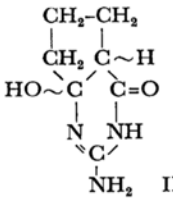
$$RCHO \xrightarrow{Ag^+} RCOOH \quad (4)$$

Scheme 5

In a similar manner, guanidine reacts with CHD in basic solutions to produce, though in low yields, 2-amino-4-oxo-1,3-diazaspiro[4.4]non-1-ene (VIII) and 1-hydroxy-3-amino-5-oxo-2,4-diazabicyclo-[4.3.0]non-2-ene (IX).



VIII



IX

Here also both the compounds have almost the same UV spectra, VIII, $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 226 (7300): IX, $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 227 (9900), showing that one, formed with the loss of two atoms of hydrogen, does not have an isocytosine chromophore.

Though the mass spectrum of IX exhibits the expected parent peak (*m/e* 169), the strength is found to vary markedly depending upon the measurement conditions; however, the peak corresponding to $M^+ - H_2O$ (*m/e* 151) is always predominant.

Thus, the reaction of CHD with arginine and guanidine reported here will, we believe, offer a way of approaching the novel reaction of $\alpha$-diketones.

## Experimental

**Reaction of Arginine with Cyclohexanedione (CHD) in 0.2N NaOH.** The reaction was carried out in almost the same way as that described by Toi *et al.*[1] To a solution of 900 mg of arginine in 100 m*l* of a 0.2N sodium hydroxide solution, there was added a 670-mg portion of CHD. After the solution had been left at room temperature for 4 hr, it was charged on a column of IR 120 (3×60 cm) and developed with 1N aqueous ammonia. The ninhydrin positive fractions were collected, and, after the water and ammonia had been evaporated under reduced pressure, the residue was chromatographed on cellulose (2×90 cm) and developed with a mixture of butanol, acetic acid, and water (4: 1: 2) or with a mixture of propanol, pyridine, acetic acid and water (15: 10: 3: 10). After repeated chromatography, five compounds, each showing a single spot on TLC, were obtained. The yields of Compounds **1** to **5**, corresponding to the spots from **1** to **5** in Fig. 1, were 600 mg, 2 mg, 7 mg, 2 mg, and 30 mg respectively. Compound **1** had already been established to be $N^5$-(4-oxo-1,3-diazaspiro-[4.4]non-2-ylidene)-L-ornithine (I), and Compound **4** was found to be arginine.

Though, under these experimental conditions, the yield of Compound **3** was too low to collect enough of a sample to study the structure, the same compound was also obtained as a major product under other conditions where the concentrations of sodium hydroxide were lower (see below).

The structure of Compound **2**, mp 172—177°C (dec), and Compound **5**, mp 205—210°C (dec), are now under investigation.

**Reaction of Arginine with CHD in 0.05N NaOH: $N^5$-(1-Hydroxy-5-oxo-2,4-diazabicyclo[4.3.0]non-2-ylidene)ornithine (III).** The reaction was carried out according to the same procedure as has been described above except that the concentration of sodium hydroxide was decreased to 0.05N. The yield of Compounds **1**, **2**, and **3**, after repeated chromatography, were 50, 100, and 130 mg respectively. Compound **3**, which was identified as $N^5$-(1-hydroxy-5-oxo-2,4-diazabicyclo-[4.3.0]-non-2-ylidene)ornithine (III) on the basis of the data to be presented below, was obtained as a colorless precipitate from water and ethanol. It showed no definite mp and gradually decomposed at 230—270°C; $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 217 (17100); NMR ($D_2O$) 1.87 (broad 10H) 3.35 (t 2H $J$=6 cps) 3.74 (t 1H $J$=5.5 cps) 4.10 (broad t 1H $J$=6 cps).

Found: C, 47.71; H, 7.05; N, 18.60%. Calcd for $C_{12}H_{20}N_4O_4 \cdot H_2O$: C, 47.67; H, 7.33; N, 18.53%.

On heating at 140°C for 3 hr, 5.54% of the total weight was lost, corresponding to a theoretical value of 5.97% based on one mole of the water of crystallization.

Found: C, 50.30; H, 6.93; N, 19.17%. Calcd for $C_{12}H_{20}N_4O_4$: C, 50.69; H, 7.09; N, 19.71%.

The trifluoroacetylated methyl ester of IV did not show $M^+$; $(M-H_2O)^+$ 376.1327 (Calcd for $C_{15}H_{19}N_4O_4F_3$, 376.1358).

**Reaction of Arginine with CHD at pH 11.** To 10 m*l* of an aqueous solution containing 1.2 g of arginine, there were added, drop by drop, 760 mg of CHD dissolved in 10 m*l* of methanol. The consumption of arginine was followed by a decrease in the pH; the pH (10.5; initial) was kept almost constant (pH 10.5—11.0) throughout the addition by adding sodium hydroxide. After the solution had then been left at room temperature for 4 hr, the methanol was removed by evaporation and the residual aqueous solution was submitted to the procedure described in the first section.

The spectrophotometric determination of the ninhydrin-positive spots on TLC showed that, in this experimental run, the yields of Compounds **1**, **2**, and **5** were scanty and that Compound **3** was the major product, accompanied by an almost equimolar amount of the unreacting arginine. TLC also showed the presence of another, as-yet-unidentified ninhydrin-positive compound, which, on TLC, moved slightly slower than Compound **3**.

**Reaction of Arginine with CHD at pH 11 in the Presence of Silver Nitrate.** To a mixture of 130 mg of arginine and 115 mg of silver nitrate dissolved in 10 m*l* of water, a solution of 300 mg of CHD in 10 m*l* of methanol was added drop by drop, with the pH kept at 10.5—11.0 with sodium hydroxide. TLC on cellulose showed that Compound **3** was predominant, accompanied by small amounts of Compounds **1** and **2**. Hardly any arginine was detected.

A control experiment was carried out in the absence of silver nitrate. TLC showed that the amount of Compound **3** and arginine, after the reaction was completed, was almost the same. The isolation of Compound **3** was not attempted in this experimental run.

**$N^1$-Benzoyl-$N^5$-(5,6-Trimethylene-4-oxopyrimid-2-yl)ornithine Ethyl Ester and $N^5$-(5,6-Trimethylene-4-oxopyrimid-2-yl)ornithine (IV).** To a solution of 343 mg ($10^{-3}$M) of benzoyl-L-arginine ethyl ester hydrochloride dissolved in 10 m*l* of alcohol, an equimolar amount of sodium alcoholate (23 mg of sodium in 1 m*l* of alcohol) was added; the solution was then left in a refrigerator for 5 hr. The precipitate of sodium chloride was removed by filtration, and 150 mg of 2-ethoxycarbonyl-cyclopentanone were added. After the solution had been refluxed for 5 hr, water was added and the product, $N^1$-benzoyl-$N^5$-(5,6-trimethylene-4-oxopyrimid-2-yl)ornithine ethyl ester (the benzoylated ethyl ester of IV), was extracted with ethyl acetate. After the solvent had been evaporated, the residue was crystallized from benzene and ligroin; yield, 15 mg; mp 138—140°C; $M^+$ 398.1980 (Calcd for $C_{21}H_{26}N_4O_4$, 398.1954); $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 229 (18300) 293 (8700).

$N^5$-(5,6-Trimethylene-4-oxopyrimid-2-yl)ornithine (IV) was obtained by the hydrolysis of the benzoylated ethyl ester of IV in a 2N hydrochloric acid solution, it being heated at 105°C for 20 hr. The hydrolysate from 10 mg of the benzoylated ethyl ester of IV was chromatographed on cellulose (2×90 cm) and the UV positive fractions were collected. Chromatography was repeated until a single spot, detected by both the ninhydrin reaction and UV absorption, was observed on TLC. After the solvent had been evaporated, the residue was precipitated from water and acetone; yield, 3 mg; it gradually decomposed over 190°C; $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 230 (8800) 292 (6100). The trifluoroacetylated methyl ester of IV showed $M^+$, 376.1340 (Calcd for $C_{15}H_{19}N_4O_4F_3$, 376.1358).

**Reaction of Guanidine with CHD: 2-Amino-4-oxo-1,3-diazaspiro[4.4]non-1-ene (VIII) and 1-Hydroxy-3-amino-5-oxo-2,4-diazabicyclo[4.3.0]non-2-ene (IX).** A mixture of guanidine hydrochloride (191 mg, $2\times10^{-3}$M) and CHD (224 mg, $2\times10^{-3}$M) dissolved in a 1N sodium hydroxide solution (10 m*l*) was left to stand at room temperature overnight, and then the sodium hydroxide was removed with IR 120, as has been described in the first section. The products were eluted from the column with 1N aqueous ammonia, and, after the ammonia and water had been evaporated under reduced pressure, the residue was submitted to partition chromatography on silicic acid (2×60 cm), being developed with butanol saturated with water. Two products, which were detected by means of a Greig-Leaback reaction (*t*-butylhypochlorite-tolidine) on TLC, were isolated.

2-Amino-4-oxo-1,3-diazaspiro[4.4]non-1-ene (VIII), eluted first from the column, was crystallized from ethanol and benzene; yield, 27 mg; mp 260—265°C; $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 226 (7300); $M^+$ 153.0911 (Calcd for $C_7H_{11}N_3O$, 153.0902).

The NMR spectrum showed a broad methylene signal at $\delta$ 1.2. The IR spectrum showed the following intense bands ($cm^{-1}$): 1632, 1585, 1487, 1335, 1320.

Found: C, 51.94; H, 7.32; N, 25.49%. Calcd for $C_7H_{11}N_3O\cdot\frac{1}{2}H_2O$; C, 51.83; H, 7.46; N, 25.91%.

1-Hydroxy-3-amino-5-oxo-2,4-diazabicyclo[4.3.0]non-2-ene (IX), which was eluted later, was crystallized from water; yield, 11 mg. It did not show a definite melting point; rather it gradually decomposed at 220—240°C; $\lambda_{max}^{H_2O}$ m$\mu$ ($\varepsilon$) 227 (9900); $M^+$ 169.0876 (Calcd for $C_7H_{11}N_3O_2$, 169.0851), $(M-H_2O)^+$ 151.0706 (Calcd for $C_7H_9N_3O$, 151.0746). The IR spectrum showed the following intense bands ($cm^{-1}$): 1675, 1662, 1612, 1490.

Found: C, 49.67; H, 6.34; N, 24.75%. Calcd for $C_7H_{11}N_3O_2$: C, 49.63; H, 6.55; N, 24.84%.

**Mass-spectrum Measurements.** A mass spectrometer of the JMS-01SG model of the Japan Electron Optics Laboratory, Ltd., was used. To obtain volatile samples, milligram amounts of I, III, and IV were methylated and successively trifluoroacetylated following the method described by McLafferty.[5] The products were, without further purification, dissolved in a small amount of acetone and charged into capillary tubes. The acetone was removed in the spectrometer under reduced pressure, and the spectra were taken under a direct-inlet system.

The author wishes to thank Dr. Saburo Suzuki, the chief of the Laboratory of Antibiotics, of this Institute, for his encouragement.

5) M. Senn, R. Venkataraghavan and F. W. McLafferty, *J. Am. Chem. Soc.*, **88**, 5593 (1966).