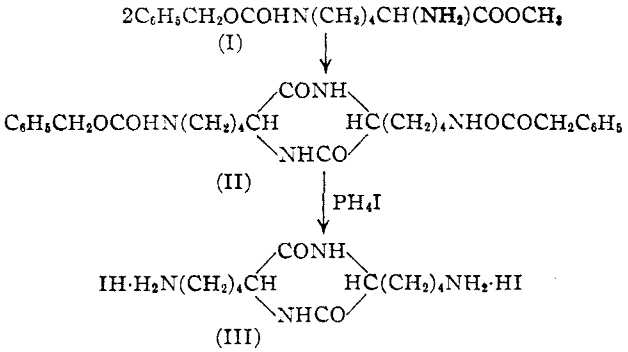
[CONTRIBUTION FROM THE LABORATORY OF HIGH MOLECULAR CHEMISTRY, THE HEBREW UNIVERSITY]

Synthesis of Lysine Anhydride

BY EPHRAIM KATCHALSKI, ISAAC GROSSFELD AND MAX FRANKEL

In connection with work on polylysine,[1] it became necessary to study the properties of lysine anhydride and its derivatives. A preparation under this name was first described by Fischer and Suzuki[2] who obtained it by heating *dl*-lysine methyl ester to 100° and assumed it to have the diketopiperazine structure corresponding to (III). Recently Adamson[3] has shown that the substance obtained by Fischer and Suzuki contained at least 40% of the volatile intramolecular anhydride of lysine, *dl*-3-aminohomopiperidone, while the remainder appeared to consist of non-volatile lysyl peptides. It was therefore decided to synthesize the lysine anhydride corresponding to formula (III) by a method which would leave no doubt as to the constitution of the product obtained.

In order to prevent intramolecular anhydridization, the ε-amino group of lysine was masked by Bergmann's method of introducing the carbobenzoxy group. ε-Carbobenzoxy-*l*-lysine methyl ester (I) was liberated from the corresponding hydrochloride[4] and was converted to ε,ε'-dicarbobenzoxylysine anhydride (II) by heating to 100°. The two carbobenzoxy groups of (II) were removed by reduction with phosphonium iodide according to the procedure used by Harington and Mead,[5] since attempted reductions by the usual catalytic method were unsuccessful. From the dihydriodide (III) thus obtained the corresponding picrate, picrolonate and dihydrochloride were prepared.



$$2C_6H_5CH_2OCOHN(CH_2)_4CH(NH_2)COOCH_3$$

(I)

$$\downarrow$$

$$C_6H_5CH_2OCOHN(CH_2)_4CH\begin{matrix}\diagup CONH \diagdown\\ \diagdown NHCO \diagup\end{matrix}HC(CH_2)_4NHOCOCH_2C_6H_5$$

(II)

$$\downarrow PH_4I$$

$$IH{\cdot}H_2N(CH_2)_4CH\begin{matrix}\diagup CONH \diagdown\\ \diagdown NHCO \diagup\end{matrix}HC(CH_2)_4NH_2{\cdot}HI$$

(III)

In view of the fact that Fischer and Suzuki did not obtain the lysine anhydride corresponding to (III) from lysine methyl ester by the usual procedure it seems appropriate to summarize the considerations which support the diketopiperazine structure of the substance described here.

By masking the ε-amino group of lysine methyl ester, the possibility of intramolecular anhydridization between the carboxyl and ε-amino groups of the carbobenzoxylysine methyl ester (I) was eliminated. A proof that under the experimental conditions used cyclization takes place between two molecules of (I) leading to ε,ε'-dicarbobenzoxylysine anhydride, is afforded by the molecular weight determination of the product of anhydridization. A value of 544 was found in agreement with the calculated value of 524 for ε,ε'-dicarbobenzoxylysine anhydride.

Formula (III) was assumed to represent the product obtained after the reductive elimination of the carbobenzoxy groups from (II), since the possibility of the diketopiperazine structure being affected by the treatment with phosphonium iodide appears very remote. Formula (III) is further supported by the analytical figures obtained (carbon, hydrogen, total nitrogen, amino nitrogen and iodine) and by the fact that the product gave a positive Abderhalden test with alkaline solution of picric acid.

Experimental

Preparation of ε-Carbobenzoxy-*l*-lysine Methyl Ester.—Four grams of ε-carbobenzoxy-*l*-lysine methyl ester hydrochloride prepared according to Bergmann, Zervas and Ross[4] was suspended in about 50 ml. of dry ether. A stream of dried ammonia was then passed through the suspension for one to two hours. Throughout this process, the mixture was cooled in a freezing mixture and was shaken from time to time. The ethereal solution was filtered, dried over anhydrous sodium sulfate and the ether evaporated off *in vacuo*. In order to remove traces of ammonia from the residual colorless liquid, the latter was redissolved in ether and the solvent removed *in vacuo* at room temperature. This process was repeated. A clear viscous liquid remained, which on analysis proved to be the free ester; yield 2.5 g., 70% of the theoretical.

Anal. Calcd. for $C_{15}H_{22}O_4N_2$: CH_3O, 10.5. Found: CH_3O, 10.4.

The free ester dissolves readily in ether, alcohol and water; its aqueous solution shows a strong alkaline reaction. On heating at 100° it decomposes as described below.

ε,ε'-Dicarbobenzoxylysine Anhydride.—Two grams of ε-carbobenzoxy-*l*-lysine methyl ester was heated at 100° for three days. During this time the viscous liquid gradually solidified. The residual solid was washed with ether until it showed no alkaline reaction. The residue was recrystallized from boiling absolute alcohol, when colorless needles of ε,ε'-dicarbobenzoxylysine anhydride were obtained; yield 1.2 g., 67% of the theoretical. For analysis the substance was again recrystallized from boiling absolute alcohol, m. p. 203°.

Anal. Calcd. for $C_{28}H_{36}O_6N_4$: C, 64.1; H, 6.9; N, 10.7. Found: C, 64.0; H, 7.0; N, 10.7.

ε,ε'-Dicarbobenzoxylysine anhydride is soluble in acetic acid, pyridine and hot alcohol. It is slightly soluble in hot acetone and insoluble in ether, chloroform and water.

The molecular weight of the ε,ε'-dicarbobenzoxylysine

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(1) Katchalski, Grossfeld and Frankel, unpublished.

(2) Fischer and Suzuki, *Ber.*, **38**, 4173 (1905).

(3) Adamson, *J. Chem. Soc.*, 39 (1943).

(4) Bergmann, Zervas and Ross, *J. Biol. Chem.*, **111**, 245 (1935).

(5) Harington and Mead, *Biochem. J.*, **29**, 1603 (1935).

anhydride was determined in camphor by Rast's method. Molecular weight calcd. for $C_{28}H_{36}O_6N_4$: 524.3. Found: mol. wt., 544.0.

Lysine Anhydride Dihydriodide.—One gram of ε,ε'-dicarbobenzoxylysine anhydride was dissolved in 50 ml. of glacial acetic acid and the solution kept at 50° while a stream of dry hydrogen was passed through it; about 3–4 g. of phosphonium iodide was added in portions of about 1 g. during one and one-half to two hours. The end of the reduction was indicated by the ceasing of carbon dioxide evolution. A voluminous precipitate accumulated at the bottom of the reaction flask as the phosphonium iodide disappeared. At the end of the reduction the clear liquid was decanted and the precipitate washed several times with dry ether, and dissolved in the minimum quantity of water to decompose the excess phosphonium iodide. (When large amounts of water were used, the subsequent addition of alcohol and ether caused the lysine anhydride dihydriodide to separate out as an oily mass.) The aqueous solution was mixed with a few ml. of absolute alcohol, and the anhydride dihydriodide precipitated by the addition of 50 ml. of ether. After standing overnight in the ice box, the solvent was decanted from the crystalline precipitate and the latter dissolved in the minimum quantity of water. The aqueous solution was filtered and evaporated to dryness in a vacuum desiccator over sulfuric acid and sodium hydroxide. The solid residue was washed thoroughly with dry ether and dried *in vacuo*.

On analysis it proved to be pure lysine anhydride dihydriodide and was obtained in almost quantitative yield.

Anal. Calcd. for $C_{12}H_{26}O_2N_4I_2$: C, 28.1; H, 5.1; N, 10.9; amino N, 5.5; I, 49.6. Found: C, 28.1; H, 5.0; N, 10.8; amino N, 5.3; I, 50.0.

The highly hygroscopic lysine anhydride dihydriodide is very soluble in water but insoluble in organic solvents. On heating its aqueous solution with an alkaline solution of picric acid a deep red coloration was obtained indicating the presence of a diketopiperazine structure. The ninhydrin reaction was negative.

Lysine Anhydride Picrolonate.—Two hundred mg. of lysine anhydide dihydriodide was dissolved in 3 ml. of water and poured into a hot saturated aqueous solution containing about 400 mg. of picrolonic acid. A voluminous yellow precipitate separated out on cooling. After standing overnight in the ice box, the precipitate of lysine anhydride picrolonate was filtered and purified by dissolving in alcohol and precipitating by ether; yield 240 mg., 78% of the theoretical.

Anal. Calcd. for $C_{32}H_{40}O_{12}N_{12}$: C, 48.9; H, 5.1; N, 21.4. Found: C, 49.0; H, 5.3; N, 21.4.

On rapid heating, the picrolonate softens at 230° and decomposes at 235–237°.

It dissolves only sparingly in cold water but fairly readily in hot water. It is soluble in alcohol but insoluble in benzene and ethyl acetate.

Lysine Anhydride Picrate.—Five hundred mg. of lysine anhydride dihydriodide was dissolved in 10 ml. of water and 1 g. of solid picric acid was added. The mixture was heated until a clear solution was obtained and then allowed to cool down to room temperature. After standing overnight in the ice box, the yellow picrate was filtered off and washed several times with ether; yield 680 mg., 97% of the theoretical. For analysis the picrate was recrystallized from water.

Anal. Calcd. for $C_{24}H_{30}O_{16}N_{10}$: C, 40.3; H, 4.2; N, 19.6. Found: C, 40.3; H, 4.3; N, 19.6.

On rapid heating the substance liquefies at about 240° and explodes at 243–245°. It is soluble in hot water and in methyl and ethyl alcohol, but insoluble in ether and benzene.

Lysine Anhydride Dihydrochloride.—1.25 ml. of 1 *N* hydrochloric acid was added to 300 mg. of lysine anhydride picrate suspended in 5 ml. of water. The mixture was shaken several times with ether to remove the picric acid liberated. An equal volume of alcohol was then added to the aqueous solution and the mixture dried at room temperature in a vacuum desiccator over sulfuric acid and sodium hydroxide. The remaining hydrochloride was washed several times with dry ether and dried *in vacuo*. The yield was quantitative.

Anal. Calcd. for $C_{12}H_{26}N_4Cl_2$: Cl, 21.6. Found: Cl, 21.8.

The dihydrochloride is very soluble in water and gives a strong red coloration when heated with an alkaline solution of picric acid. The ninhydrin reaction is negative. The dihydrochloride is insoluble in organic solvents.

Summary

ε,ε'-Dicarbobenzoxylysine anhydride (II) was prepared by heating ε-carbobenzoxy-*l*-lysine methyl ester.

The two carbobenzoxy groups of (II) were removed by reduction with phosphonium iodide and lysine anhydride dihydriodide (III) was obtained.

From (III) the corresponding picrolonate, picrate and dihydrochloride were prepared.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Action of Sulfuric Acid on Tertiary Carboxylic Acids[1]

BY E. CAMPAIGNE[2] AND C. M. SUTER[3]

Bistrzycki[4] investigated the cleavage of carbon monoxide from tertiary carboxylic acids in concentrated sulfuric acid. The product, yield, and conditions of his experiments are summarized in Table I.

Böeseken[5] obtained a product similar to that isolated by Bistrzycki by heating trimethylacetyl chloride to 100° in concentrated sulfuric acid. By stopping the reaction at 50° he was able to detect a small amount of isobutylene polymer.

TABLE I

	Starting material	Product	Yield, %	Temp., °C.
1	$(C_6H_5)_3CCOOH$	$(C_6H_5)_3COH$	90	25
2	$(C_6H_5)_2(CH_3)CCOOH$	$(C_6H_5)_2C{=}CH$	85	25
3	$C_6H_5(CH_3)_2CCOOH$	$C_9H_9SO_3H^a$	51	70
4	$(CH_3)_3CCOOH$	$C_4H_6S_2O_6H_2^a$	35	110

[a] These products were analyzed, but the structures were not determined.

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[1] Presented at the meeting of the American Chemical Society, Memphis, Tennessee, April, 1942.

[2] Post-doctorate fellow 1941–1942; present address: Chemistry Department, Indiana University, Bloomington, Indiana.

[3] Present address: Winthrop Chemical Company, Inc., Rensselaer, N. Y.

[4] Bistrzycki and Mauron, *Ber.*, **40**, 4370 (1907).

[5] Böeseken, *Rec. trav. chim.*, **29**, 85 (1910).