TABLE I

THERMAL STABILITY EXPERIMENTS ON CRYSTALLINE THALLOUS HYPOPHOSPHITE

Series	Initial weight sample (g.)	Net weight change in mg. after 24 hr. heating at the indicated temperature (°C.)								
		50°	65°	75°	80°	90°	90°—48 hr. weighing periods			
I	0.843	−0.2	−0.4	+0.2	+2.7	$+1.8^a$	+2.4	+2.4	$+3.4^b$	+5.0
		Net weight change in mg. after 48 hr. heating[c] at the indicated temperature (°C.)								
IA	0.847				80°	89°	95°	95°		
					−0.2	−0.2	−0.2	−1.3		
		Net weight change in mg. after heating[d] at 95° for the indicated time intervals								
II	0.993		3 hours	1 hour	2 hours	3 hours	3 hours			
			−2.0	−0.1	−0.1	−0.2	−0.1			
		Net weight change in mg. after 3 hours heating at the indicated temperature (°C.)								
III	0.672				95°	95°	95°	100°	100°	
					−0.6	−0.2	−0.2	−0.5	−0.8	
		Net weight change in mg. after heating as follows:								
IV[e]	1.137		95° for 3 hr.	95° for 3 hr.	100° for 2 hr.	100° for 2 hr.				
			−0.8	−0.2	−0.3	−0.2				

[a] Partial melting began at this temperature. [b] Complete liquefaction. [c] Series IA was run as follows: The sample was first evacuated to a pressure of 10^{-5} mm. The system was then thoroughly flushed out with argon, re-evacuated, the sample closed off from the pump and heated. At the end of the heating period, the system was re-evacuated, the heater removed, and the sample allowed to cool while connected to the pump. [d] In this series, the salt was neither ground up nor vacuum dried before putting it in the vacuum line. [e] In this series, salt, which had been directly crystallized from water alone, was used; in all others, salt which had been crystallized from isopropyl alcohol was used.

behavior. (b) When air was excluded by evacuation or flushing with argon, neither melting nor weight *increase* was ever observed, even though the temperature was 35° above the melting point of thallous *phosphite* for considerable periods of time. This supports the conclusion drawn above concerning air oxidation of thallous hypophosphite at elevated temperatures. (c) In all cases, the weight loss observed in the first heating period was from four to five times that observed in subsequent periods. This can probably be attributed to the last traces of solvent disappearing from the salt.

In some cases, analyses were run on the samples after heating; no significant change in the thallium content was noted.

It is possible that the small, reproducible weight losses observed *after* the larger initial weight losses in Series II through IV were due to one or both of the reactions

$$5TlH_2PO_2 \longrightarrow Tl_4P_2O_7 + TlPO_3 + PH_3 + 2H_2{}^{1b}$$
$$2TlH_2PO_2 \longrightarrow Tl_2HPO_4 + PH_3$$

To test this point, one attempt was made to trap out, with liquid nitrogen, any phosphine that might be produced. No phosphine was visible in the trap, but when air was rapidly admitted to the system, a green flame appeared in the trap—such behavior is characteristic of phosphine at low pressures. Qualitative tests for phosphate were made after series IA and II. A faint positive test was observed in IA.

It is further worth noting that some of our thallous hypophosphite preparations seemed to be somewhat sensitive to light; that is to say, if allowed to stand in strong sunlight for a week or so, they began to develop the same black color noted in the heating experiments. Control samples in blackened bottles developed no color. For this reason, all experiments involving this salt at elevated temperatures were run in the dark, and the amount of exposure of the salt to light while cold was minimized as much as possible.

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Ornithine Anhydride

BY EPHRAIM KATCHALSKI AND PNINA SPITNIK[1]

In connection with a study of poly-DL-ornithine,[2] the properties of ornithine anhydride and its derivatives were investigated. Ornithine anhydride was synthesized in the manner described for lysine anhydride,[3] using δ-carbobenzoxy-DL-ornithine methyl ester as the starting material. Ornithine anhydride gives a positive picric acid test[4] and a negative biuret reaction; in its chemical properties it closely resembles lysine anhydride.

Experimental

α,δ-Dicarbobenzoxy-DL-ornithine.—Obtained in 95% yield by coupling DL-ornithine with carbobenzoxy chloride in the usual way,[5] m.p. 110–112° (from boiling benzene).[6]

It readily dissolves in ethyl acetate and boiling benzene, is slightly soluble in ether and is insoluble in water and in petroleum ether.

Anal. Calcd. for $C_{21}H_{24}N_2O_6$: C, 63.0; H, 6.0; N, 7.0. *Found:* C, 63.2; H, 6.0; N, 7.1.

δ-Carbobenzoxy-α-N-carboxy-DL-ornithine Anhydride.—The foregoing substance (10 g.) was dissolved in boiling benzene (200 ml.), the solution quickly cooled to room temperature and phosphorus pentachloride (10 g.) added. The

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(1) This paper is part of a thesis presented by Pnina Spitnik to the Hebrew University, Jerusalem, in partial fulfillment of the requirements of the degree of Ph.D.

(2) E. Katchalski and P. Spitnik, *Nature*, **164**, 1092 (1949).

(3) E. Katchalski, I. Grossfeld and M. Frankel, THIS JOURNAL, **68**, 879 (1946).

(4) E. Abderhalden and E. Komm, *Z. physiol. Chem.*, **139**, 181 (1924).

(5) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935).

(6) Optically active α,δ-dicarbobenzoxy-L-ornithine, m.p. 112–114°, has been described by R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948).

temperature was then lowered to 0° and the mixture shaken for a few minutes until practically all the phosphorus pentachloride had disappeared. The solution was filtered rapidly and the filtrate allowed to stand at room temperature for three hours. The solid which separated was filtered and washed with ice-cold benzene and petroleum ether (yield 60%). It was recrystallized from a small amount of boiling benzene, m.p. 110° with carbon dioxide evolution. The N-carboxy anhydride is soluble in ethyl acetate, acetone and boiling benzene, is slightly soluble in ether and insoluble in petroleum ether. The purity of the substance may be tested by heating the N-carboxy anhydride with hydrochloric acid; carbon dioxide is evolved and a clear solution obtained.

Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.5; H, 5.5; N, 9.6. Found: C, 57.8; H, 5.3; N, 9.7.

δ-Carbobenzoxy-DL-ornithine Methyl Ester Hydrochloride.—Prepared from δ-carbobenzoxy-α-N-carboxy-DL-ornithine anhydride analogously to the corresponding lysine derivative.[5] It is extremely hygroscopic; m.p. 130° after drying *in vacuo* over sodium hydroxide and sulfuric acid. (Synge[6] gives m.p. 132–134° for the L-isomer.) It readily dissolves in ethyl acetate at room temperature and in warm ethanol and benzene.

Anal. Calcd. for $C_{14}H_{21}N_2O_4Cl$: C, 53.4; H, 6.7; N, 8.8; Cl, 11.2. Found: C, 53.0; H, 6.7; N, 8.8; Cl, 11.5.

δ,δ'-Dicarbobenzoxy-ornithine Anhydride.—To an ice-cold solution of δ-carbobenzoxy-DL-ornithine methyl ester hydrochloride (6.2 g.) in water (5 ml.) an equivalent amount of 2 *N* sodium hydroxide was added. The solution was extracted with ether, saturated with anhydrous potassium carbonate and extracted again. The combined ethereal extracts were dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting product was heated for 48 hours at 110° (sealed glass tube) and the solid formed washed with anhydrous ether and recrystallized from a large volume of boiling ethanol, m.p. 221°. δ,δ'-Dicarbobenzoxyornithine anhydride dissolves in hot formic and glacial acetic acids; it is precipitated by water from its solution in dimethyl formamide. The aqueous suspension of the anhydride gives a negative ninhydrin reaction.

Anal. Calcd. for $C_{26}H_{32}N_4O_6$: C, 62.9; H, 6.2; N, 11.3; mol. wt., 496. Found: C, 62.6; H, 6.2; N, 11.5; mol. wt., 538 (Rast).

Ornithine Anhydride Dihydriodide.—Obtained in 60% yield by reduction of δ,δ'-dicarbobenzoxyornithine anhydride with phosphonium iodide in glacial acetic acid.[3] It readily dissolves in water; the aqueous solution gives a strong positive picric acid test[4] and a negative biuret reaction.

Anal. Calcd. for $C_{10}H_{22}N_4O_2I_2$: C, 24.8; H, 4.6; I, 52.4; amino-N, 5.8; N, 11.6; carboxyl-N, 0.0. Found: C, 25.0; H, 5.0; I, 51.6; amino-N, 5.6; N, 11.4; carboxyl-N,[7] 0.0.

An acid hydrolysate of ornithine anhydride dihydriodide was prepared and analyzed as follows: the dihydriodide (15.6 mg.) was refluxed for 24 hours in 20% hydrochloric acid (7 ml.). The hydrolysate was neutralized with sodium hydroxide and brought to 15 ml. In 2 ml. of the final solution the amount of carboxyl-N[7] in another 2 ml. the total free amino-N was determined (Van Slyke manometric method—on shaking half an hour with nitrous acid). From the data obtained, the total amounts of carboxyl-N and amino-N in the hydrolysate were calculated. The amount of these groups per 100 mg. of starting material are given below.

Anal. Calcd. for hydrolysis of 100 mg. ornithine anhydride dihydriodide: carboxyl-N, 5.6 mg.; amino-N, 11.8 mg. Found: carboxyl-N, 5.8 mg.; amino-N, 11.6 mg.

The picrate of ornithine anhydride was prepared from an aqueous solution of ornithine anhydride dihydriodide, m.p. 250° (dec.); slightly soluble in acetone and hot ethanol, insoluble in ether.

Anal. Calcd. for $C_{22}H_{26}N_{10}O_{16}$: C, 38.5; H, 3.8; N, 20.4. Found: C, 38.7; H, 4.0; N, 20.8.

The dihydrochloride was prepared from the picrate in the usual way and purified by precipitation from a concentrated aqueous solution with absolute ethanol.

Anal. Calcd. for $C_{10}H_{22}N_4O_2Cl_2$: C, 39.8; H, 7.3; N, 18.6; Cl, 23.7; amino-N, 9.3. Found: C, 39.5; H, 7.4; N, 18.6; Cl, 24.0; amino-N, 9.3.

The flavianate was obtained from an ethanolic solution of the dihydriodide, m.p. 220° (dec.).

Anal. Calcd. for $C_{30}H_{32}N_8O_{18}S_2$: C, 42.5; H, 3.8; N, 13.1; S, 7.5. Found: C, 42.0; H, 4.0; N, 12.8; S, 7.1.

The **picrolonate** was obtained from a methanolic solution of the dihydriodide, m.p. 272° (dec.).

Anal. Calcd. for $C_{30}H_{38}N_{12}O_{12}$: C, 47.6; H, 5.0; N, 22.1. Found: C, 48.0; H, 5.1; N, 21.8.

δ,δ'-Dibenzylidene Ornithine Anhydride.—Ornithine anhydride dihydriodide (0.5 g.) in water (10 ml.) was brought to *p*H 9 by means of 1 *N* sodium hydroxide and shaken with benzaldehyde (0.5 g.). The benzylidene derivative separated at once in fine crystals. It was filtered, washed with ice-water, methanol and ether and recrystallized from boiling methanol, m.p. 190°.

Anal. Calcd. for $C_{24}H_{28}N_4O_2$: C, 71.2; H, 7.0; N, 13.8. Found: C, 71.0; H, 7.3; N, 14.0.

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Isolation of L-Leucyl-L-proline Anhydride from Microbiological Fermentations

BY JAMES L. JOHNSON, WILLIAM G. JACKSON AND THOMAS E. EBLE

In the course of investigating the culture filtrates from an unidentified *Streptomyces* species[1] a crystalline fraction was isolated which was proved to be L-leucyl-L-proline anhydride.[2,3,4] The material crystallized readily from the sirupy residue obtained by extracting the culture filtrate with chloroform, and concentrating the chloroform solution *in vacuo*.

An attempt to isolate L-leucyl-L-proline anhydride from unfermented culture medium failed; the compound, therefore, appears to be a true metabolite. These observations are of interest because the material has previously been isolated from hog adrenal cortex extracts,[3,4] and has since been isolated in these laboratories from culture filtrates of a streptomycin-producing strain of *Streptomyces griseus*, and *Aspergillus fumigatus* H-3 which also produces an agent which inhibits the action of *S. aureus* phage.[5]

The infrared absorption spectrum of the solid material mulled with liquid petrolatum, Fig. 1, was especially helpful in establishing its identity. Two strong bands at 1670 and 1635 cm.$^{-1}$, a single band at 3260 cm.$^{-1}$, the absence of a band at about 1550 cm.$^{-1}$, and the lack of strong C–O absorption in the lower frequency region (1250–1000 cm.$^{-1}$) indicated a cyclic monosubstituted and/or a disubstituted amide structure. The intact molecule gave a negative ninhydrin reaction, but after heating with 10% aqueous sodium hydroxide or with 6 *N* hydrochloric acid its hydrolysis products

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[1] The culture had been selected for study by Dr. A. J. Whiffen of these laboratories because the filtrates showed antibiotic activity against the fungal pathogens of man. A general discussion of the screening program used in the selection of the culture has been published: R. L. Emerson, A. J. Whiffen, N. H. Bohonos and C. DeBoer, *J. Bact.*, **52**, 357 (1946).

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