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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[7] D. D. Van Slyke, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 671 (1941).

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).

[2] E. Fischer and G. Reif, *Ann.*, **363**, 126 (1908).

[3] O. Wintersteiner and J. J. Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935).

[4] M. H. Kuizenga, J. W. Nelson, S. C. Lyster and D. J. Ingle, *ibid.*, **160**, 15 (1945).

[5] F. R. Hanson and T. E. Eble, *J. Bact.*, **58**, 527 (1949).

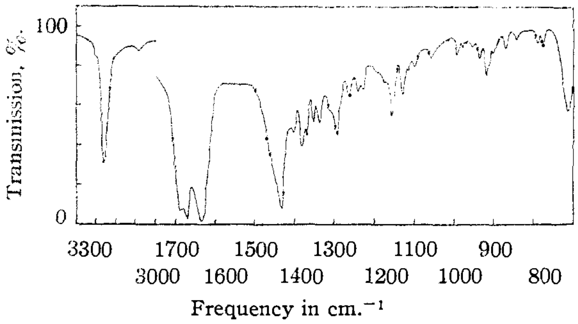

Fig. 1.

reacted with ninhydrin to give the deep purple color characteristic of α-amino acids. Paper strip chromatograms[6] showed the hydrolysis mixtures to contain leucine and proline. These data indicated the unknown to be one of the leucylproline anhydrides, and a search of the literature revealed it to be identical with the L,L-isomer described by Fischer and Reif.[2] Hydrolysis of this material with 33% aqueous sulfuric acid has been found to yield L-leucine and L-proline of good optical purity. Fischer and Reif reported hydrolysis with 20% aqueous hydrochloric acid to cause appreciable racemization of the amino acids.

Experimental

Isolation of L-Leucyl-L-proline Anhydride from the Culture Filtrates.—The culture fluid from a 100-gallon fermenter of the *Streptomyces* sp. (180 l.) was clarified by filtration. The filtrate was extracted with six 3.5-liter portions of chloroform, and the chloroform extract was concentrated *in vacuo* to about 750 ml. On standing overnight at 5°, a brown crystalline precipitate formed. It was collected by filtration and recrystallized from amyl acetate. The yield was 3.61 g. of white hexagonal plates. The sample for analysis was recrystallized from methanol; m.p. 158–161° after softening at about 145°; $[\alpha]^{21}\text{D}$ −142.4 ± 0.5° (*c*, 3.33 in ethanol). Fischer and Reif[2] reported $[\alpha]^{20}\text{D}$ −143.4°.

Anal. Calcd. for $C_{11}H_{18}O_2N_2$: C, 62.82; H, 8.62; N, 13.33; mol. wt., 210. Found: C, 62.91; H, 8.36; N, 13.48; mol. wt. (Rast), 235.

Acid Hydrolysis and Isolation of the Amino Acids.—A suspension of 3 g. of the fermentation metabolite in a mixture of 16 ml. of concd. sulfuric acid and 32 ml. of water was heated overnight on a steam-bath, then under reflux in an oil-bath for five hours. The reaction mixture was diluted to 250 ml., and solid barium hydroxide octahydrate was added to the hot solution until it was only weakly acidic. The precipitated barium sulfate was removed by filtration, and the filtrate was evaporated to dryness yielding 3.82 g. of a white crystalline residue. The residue was extracted with four 25-ml. portions of hot absolute alcohol.

L-Proline.—The alcohol extract was concentrated *in vacuo* to 10 ml. and 60 ml. of ether was added to precipitate the L-proline. The precipitate was collected by filtration, and the precipitation from absolute alcohol was twice repeated. The yield of L-proline was 1.11 g., or 70% of the theoretical amount; $[\alpha]^{22}\text{D}$ −77.7 ± 0.5° (*c*, 4.36 in water). Fischer and Zemplén[7] reported $[\alpha]^{20}\text{D}$ −79.8°.

Anal. Calcd. for $C_5H_9NO_2$: C, 52.16; H, 7.88. Found: C, 51.97; H, 7.74.

L-Leucine.—The alcohol-insoluble residue was recrystallized three times from water yielding 1.47 g. of L-leucine, 78% of the theoretical amount; $[\alpha]^{22}\text{D}$ +16.83 ± 0.4° (*c*, 5.02 in 20% HCl). Fischer[8] reported $[\alpha]^{20}\text{D}$ +17.5°.

Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.94; H, 9.99. Found: C, 54.98; H, 9.80.

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[6] Run according to the procedure of R. J. Williams and H. Kirby, *Science*, **107**, 481 (1948).

[7] E. Fischer and G. Zemplén, *Ber.*, **42**, 2989 (1909).

[8] E. Fischer, *ibid.*, **34**, 433 (1901).

Oxidation of Nitric Oxide at High Pressures of Reactants

BY HAROLD S. JOHNSTON AND LOREN W. SLENTZ

The reaction between nitric oxide and oxygen was studied in considerable detail at pressures of reactants up to one-half atmosphere at the one temperature, 25°. The method and apparatus are identical with the intermediate pressure equipment of Mills and Johnston.[1] Three series of runs were made: (1) with equal pressures of reactants, (2) with nitric oxide in tenfold excess and oxygen diluted with 9 parts of nitrogen, and (3) with oxygen in tenfold excess and nitric oxide diluted with 9 parts of nitrogen. At high pressures of reactants the rate was followed on the oscilloscope since half-times were of the order of magnitude of one-tenth second. At lower pressures of reactants, the rate was followed visually on an electronic voltmeter. The third-order rate law was observed throughout this range of conditions, and no trend in the values of the rate constants was observed in going from high to low pressures. The results are summarized in Table I.

TABLE I

THIRD ORDER RATE CONSTANTS FOR THE REACTION BETWEEN NITRIC OXIDE AND OXYGEN AT 25°

Ratio (NO)/(O₂)	Range of initial pressures of NO, mm.	Number of runs	Average rate constant, sec.⁻¹ mm.⁻² × 10⁵	Standard error of mean, sec.⁻¹ mm.⁻² × 10⁵
10	10.6 to 340	28	1.98	0.06
1	18.4 to 130	15	2.11	.05
0.1	8.1 to 43.0	20	2.02	.05
	Average of all	63	2.02	.03

The average rate constant is 7.10 × 10^9 cc.^{2} mole^{-2} sec.$^{-1}$, and the standard error of the mean is 0.12 × 10^9. This value agrees exactly with Bodenstein's[2] 7.06 × 10^9 cc.^{2} mole^{-2} sec.$^{-1}$ at 30°, and it is slightly, though perhaps not significantly, higher than Smith's[3] 6.00 × 10^9 cc.^{2} mole^{-2} sec.$^{-1}$ at 25°.

[1] Robert L. Mills and Harold S. Johnston, THIS JOURNAL, **73**, 938 (1951).

[2] M. Bodenstein, *Z. Elektrochem.*, **24**, 183 (1918); *Z. physik. Chem.*, **100**, 87 (1922).

[3] J. H. Smith, THIS JOURNAL, **65**, 74 (1943).

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Thermal Decomposition of Nitrogen Pentoxide at High Temperature

BY HAROLD S. JOHNSTON AND YU-SHENG TAO

The thermal decomposition of nitrogen pentoxide is a very extensively investigated reaction. The