to dryness. The residue was extracted with boiling ethanol, and the filtrate from the insoluble potassium sulfate was evaporated at room temperature, leaving 3.8 g. of colorless semi-crystalline residue. This was taken up in 10 ml. of hot water, which deposited 0.77 g. of well-formed crystals melting at 177–178°. Evaporation of the water from the mother liquor left a transparent gum which could not be induced to crystallize. The infrared absorption curve of the crystalline product was identical with that of an authentic specimen of 5,5-dimethylhydantoin.

2,3-Dimethyl-2,3-dinitrobutane.—This intermediate was prepared from 2-bromo-2-nitropropane and the sodium salt of 2-nitropropane by the method of Seigle and Hass.[7] In a later preparation, this method was modified as follows, so as to combine in a single operation the bromination of 2-nitropropane[8] and the Seigle and Hass condensation. A stirred and cooled mixture of 44.5 g. (0.5 mole) of 2-nitropropane and 84 ml. of 6 *N* sodium hydroxide was half brominated by the dropwise addition of 40.0 g. (0.25 mole) of bromine, and 165 ml. of ethanol was then added. The stirred solution was boiled gently under a reflux condenser for 3 hours, toward the end of which the product began to crystallize. The slurry was poured into 500 ml. of ice-water and filtered, yielding 24.7 g. of 2,3-dimethyl-2,3-dinitrobutane (56% of theoretical).

2,3-Dimethylbutane-2,3-diamine.—The dinitro compound was reduced by the method of Bewad.[9] A slurry of 17.6 g. (0.1 mole) of 2,3-dimethyl-2,3-dinitrobutane in 150 ml. of concentrated hydrochloric acid was vigorously stirred at 50–60° during the gradual addition of 75 g. of 20-mesh granular tin. The solution was boiled for 15 minutes under a reflux condenser, made strongly alkaline by the addition of sodium hydroxide, and steam distilled. All of the diamine came over in the first 350 ml. of distillate, from which the new imidazolidine derivatives could be obtained directly by treatment with carbon disulfide or with phosgene, as described below. In order to isolate the diamine as the free base, the addition of about 100 g. of solid sodium hydroxide to the distillate resulted in the separation of most of it in fairly pure form as a liquid layer. The diamine could also be isolated as its oxalate by neutralizing the distillate with 1 *N* oxalic acid, which caused the rapid formation of fine colorless crystals melting at 323–324°. The yield of oxalate, which was nearly insoluble in alcohol as well as in water, was 15.7 g. (76%). *Anal.*[10] Calcd. for $C_8H_{18}N_2O_4$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.79; H, 9.10; N, 13.44.

2-Thiono-4,4,5,5-tetramethylimidazolidine (I).—The distillate from the reduction of 17.6 g. of 2,3-dimethyl-2,3-dinitrobutane was treated with 10 g. of carbon disulfide, and the mixture was boiled under a reflux condenser for 2 hours, during which hydrogen sulfide was eliminated and a crystalline product began to separate. The resulting slurry was evaporated to about half its initial volume, cooled, and filtered, yielding 9.8 g. of colorless 2-thiono-4,4,5,5-tetramethylimidazolidine (62% of theoretical based on the dinitro compound). The product, recrystallized from ethanol, melted at 252–253°; no decomposition occurred, as shown by the fact that the melt was allowed to solidify several times and could be remelted without change in appearance or behavior. *Anal.* Calcd. for $C_7H_{14}N_2S$: C, 53.12; H, 8.92; N, 17.70; S, 20.26. Found: C, 53.05, 53.17; H, 8.83, 8.81; N, 17.63, 17.50; S, 20.04, 20.33.

It is of interest to note that melting point depressions of only 2–4° were observed when 2-thiono-4,4,6-trimethyl-1,2,3,4-tetrahydropyrimidine melting at 254–255° was mixed in proportions varying from 1:3 to 3:1 with the new compound. 2-Thiono-4,4,5,5-tetramethylimidazolidine appears to be devoid of mercaptan-like properties, as shown by its insolubility in alkali and its failure to undergo oxidation with iodine.

4,4,5,5-Tetramethyl-2-imidazolidone.—A total of 1140 ml. of 0.125 *M* potassium permanganate, added in small portions with stirring and toward the end with heating, was required to oxidize 9.5 g. (0.06 mole) of 2-thiono-4,4,5,5-tetramethylimidazolidine. The colorless filtrate from the precipitated manganese dioxide was neutralized with sulfuric acid and evaporated to incipient crystallization. The slurry was cooled and filtered, yielding 7.23 g. of colorless 4,4,5,5-tetramethyl-2-imidazolidone (84% of theoretical). The compound melted at 288–289°, unchanged after recrystallization from 110 ml. of ethanol. *Anal.* Calcd. for $C_7H_{14}N_2O$: C, 59.12; H, 9.93; N, 19.70. Found: C, 59.19, 59.12; H, 9.94, 9.86; N, 19.68, 19.97.

As confirmation of the structure of this compound, one-half of the distillate from the reduction of 17.6 g. of 2,3-dimethyl-2,3-dinitrobutane was treated with 20 ml. of 5 *N* sodium hydroxide and brought to slight acidity by bubbling phosgene into the stirred solution at a moderate rate. After evaporation to dryness, the residual solid was extracted with 100 ml. of boiling ethanol. Evaporation of the extract left a crystalline residue weighing 3.57 g. (50%). Recrystallization from water gave pure 4,4,5,5-tetramethyl-2-imidazolidone melting at 288–289°.

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Application of the Japp–Klingemann Reaction.[1] A New Synthesis of Ornithine

BY D. SHAPIRO AND R. A. ABRAMOVITCH

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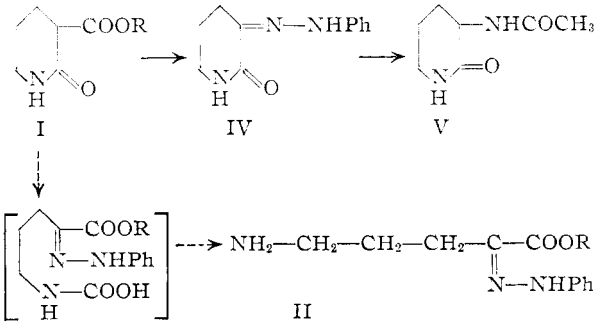
Manske and Robinson[2] showed that the reaction of ethyl cyclopentanone-2-carboxylate with diazotized aniline led to a ring opening with consequent formation of the half ester phenylhydrazone of α-ketoadipic acid. In the course of a study of the Japp–Klingemann reaction with compounds of this type it occurred to us that a similar reaction with 3-carbethoxy-2-piperidone(I)[3] might cause an analogous ring opening, thus giving rise to the phenylhydrazone (II). In fact, the Japp–Klingemann reaction of the ester (I, R = Et) with diazotized aniline gave a tar which did not contain any II or the possible corresponding lactam IV, neither was any carbon dioxide evolution observed. If, however, the ester was first hydrolyzed to the acid (I, R = H), then coupling with benzenediazonium chloride took place readily giving IV in moderate yields, and this, in turn, gave 3-acetamido-2-piperidone (V) on reductive acetylation. Since V has already been hydrolyzed to ornithine[4] these reactions provide a new approach to the latter amino acid.



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Experimental

Piperid-2,3-dione-3-phenylhydrazone.—A solution of 8.5 g. of 3-carbethoxy-2-piperidone in 100 cc. of water containing 3 g. of potassium hydroxide was kept at 30° overnight and then brought to *p*H 4–5 by the addition of dilute hydrochloric acid. It was then treated with stirring at 0° with a partially neutralized solution (150 cc.) of benzenediazonium chloride prepared from 4.85 g. of aniline and 3.75 g. of sodium nitrite, and after stirring for a few minutes the *p*H of the solution was brought to 5–6 by the addition of 40 cc. of a 45% aqueous solution of sodium acetate. A yellow turbidity formed immediately. Stirring at 5–10° (internal temperature) was continued for 5 hours, the solid filtered, washed with water and a small amount of alcohol and dried; yield 8.8 g. Recrystallization from aqueous alcohol gave pale cream-colored needles, m.p. 244–245° dec.

Anal. Calcd. for $C_{11}H_{13}ON_3$: C, 65.0; H, 6.45; N, 20.7. Found: C, 65.2; H, 6.6; N, 21.2.

3-Acetamido-2-piperidone.—1.92 g. of the above hydrazone in 30 cc. of warm glacial acetic acid was slowly added at 20–25° to a stirred suspension of 5 g. of zinc dust in 15 cc. of glacial acetic acid containing 8 cc. of acetic anhydride. Stirring at room temperature was continued for 0.5 hour, the mixture was then filtered, and the filtrate diluted with 20 cc. of water, allowed to stand for 1 hour and evaporated to dryness *in vacuo*. The residue was extracted repeatedly with ether to remove acetanilide, the residue extracted with hot chloroform, the extract taken to dryness and the residue again extracted with ether. The final residue was dissolved in hot chloroform, filtered from some insoluble material, concentrated and treated with ether and a little acetone until a cloudiness appeared. After some time the crystalline solid was filtered, washed with acetone, and dried; yield 0.55 g., m.p. 181–184°. Recrystallization from chloroform–ether gave colorless needles, m.p. 184–185° (Bergmann and Koster[3] give m.p. 187–188°).

Anal. Calcd. for $C_{17}H_{12}O_2N_2$: C, 53.8; H, 7.7; N, 17.9, Found: C, 53.9; H, 7.7; N, 17.4.

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A Simple Synthesis of Isotopic Citrulline and Two of Its Homologs

BY LLOYD H. SMITH, JR.[1]

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A standard synthesis of citrulline from urea and copper chelated ornithine was developed by Kurtz[2] and recently was described in detail.[3] This method gives a good yield of citrulline from ornithine (approximately 60–70% of theoretical) but a low yield from urea (*ca.* 15%) because of the excess of urea required for optimal synthesis by the fusion procedure. Changing the urea to ornithine ratio to equimolar amounts gives a maximal yield of citrulline from urea of about 25%.[4] Recent interest in citrulline has been largely directed toward the reactions of its carbamyl group in arginine formation,[5] degradation by citrullinase,[6] and conversion to pyrimidines *via* ureidosuccinic acid.[7] A simple synthesis of citrulline, especially adapted to isotopic labeling of the carbamyl group, has been developed by the condensation of copper chelated ornithine with cyanate. Yields of free citrulline of 55–65% based on either ornithine or cyanate were obtained. This method would seem to offer the following advantages: (1) a high yield of carbamyl labeled citrulline from the isotopic precursor, about 2.5 times that obtained by the Kurtz procedure; (2) simplicity and rapidity, the use of a sealed tube is avoided and at 100° the reaction is complete in 20 minutes. (3) more complete conversion of copper citrulline tc free citrulline.

By analogous reactions carbamyl labeled 1-homocitrulline-C^{14} (ϵ-carbamyllysine) and 1-α-amino-γ-carbamidobutyric acid-C^{14} were synthesized and found to be inactive as substrates for citrullinase and for conversion to compound X/carbamyl phosphate. Both compounds were weak inhibitors (inhibition indices of about 10 for 50% inhibition) of the release of CO_2 from 1-citrulline-C^{14} by citrullinase, but did not inhibit compound X/carbamyl phosphate formation at this concentration. Ornithine was found to be an unusually potent inhibitor of compound X/carbamyl phosphate formation from citrulline, which will be detailed elsewhere.[7] The previously described inhibition of citrullinase by ornithine[6] was confirmed.

Experimental

Synthesis of Citrulline.—410 mg. (2.0 mmoles) of *l*(+)-ornithine dihydrochloride was chelated with cupric carbonate as described by Kurtz[8] and it reacted with 178 mg. (2.2 mmoles) of potassium cyanate at 37° in a final volume of about 3 cc. Precipitation of copper citrulline began within 2 hours. (A time study of yield based on ornithine revealed: 24 hr., 68%; 48 hr., 74.5%; 72 hr., 77%; 96 hr., 78.5%; 120 hr., 80%). Yield of crude copper citrulline was 312 mg. (75% based on ornithine). The range of yield was 72–82% on repeated tests. The copper citrulline was brought into solution with 0.8 cc. of 6 *N* HCl, diluted with 8 cc. of water and treated with H_2S for 10–15 minutes. CuS was removed by filtration, and chloride removed with a Dowex 2-acetate column (1 × 8 cm.). Free citrulline was obtained by concentration of the eluate *in vacuo* and the addition of absolute ethanol as previously described.[2,3] Yield was 210 mg. (60% based on ornithine, 80% from the copper complex); m.p. 218–219° dec. (uncor.). The range in yield of free citrulline based on ornithine was 55–65% with a range in melting points (dec.) of 214–228° for this unrecrystallized product. Mixed melting points with authentic citrulline were not depressed; $(\alpha)^{22}D$ +21° (5% solution in 1 *N* HCl), reported $[\alpha]^{25}D$ +21°.[3] Paper chromatography with phenol–water 80:20 revealed a single ninhydrin spot with an R_f 0.62.[9] By recrystallization from aqueous alcohol[3] needles were obtained with m.p. 230–232° dec., but the unrecrystallized product was adequate for biological purposes.

In an alternate method the copper ornithine–cyanate solution was heated in a boiling water-bath for 20 minutes. The yield of copper citrulline by this method had a range of 70–78% based on ornithine. The rapidity and ease of this method makes it the preferable one for non-isotopic synthesis of citrulline.

For the synthesis of citrulline-C^{14}, isotopic cyanate was formed by the fusion of urea and anhydrous potassium carbonate, as described by Williams and Ronzio.[10] 120 mg. (2.0 mmoles) of urea-C^{14} was intimately ground with 166 mg. (1.2 mmoles) of potassium carbonate and transferred to a Pyrex test-tube. Better yields were obtained by beginning the fusion at 130–140° in a metal-bath, gradually in-

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