

THE PREPARATION OF CITRULLINE BY HYDROLYSIS OF ARGININE

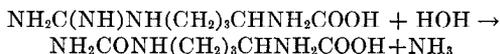
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The need for 50 gm. of citrulline for the thermal data being compiled in this laboratory (1, 2) led to the search for a suitable method of preparation of this compound. Of the methods available, the most practical were Kurtz's ingenious three reaction degradation of arginine (3), and Wada's tryptic digestion of casein (4). Each of these authors, however, reported the preparation of less than 2 gm. of citrulline, and neither method is of the type to lend itself readily to the processing of larger batches. The necessity for troublesome barium hydroxide and sealed tube manipulations in one case, and a 3 week incubation in the other, increased the desirability of a rapid and simple process.

Although the biological conversion of arginine to citrulline is well known (5, 6), these reactions are inconvenient as preparative procedures. The alkaline hydrolysis of arginine has been shown to give ornithine (7, 8), urea (9), and carbon dioxide and ammonia (10). The suggestion that citrulline might similarly be obtained has been offered, and at least one such attempt has been recorded (3, 5, 11). The hydrolysis of arginine may be represented as follows:



The present author has, under proper conditions, consistently isolated copper citrullinate from the alkaline hydrolysis of arginine solutions. This discrepancy with former work may be due to the fortunate finding of an effective means of isolation. A more likely explanation, however, is that the previous authors, in using a large

molar excess of alkali, caused rapid decomposition of any citrulline formed. To test this, in the present investigation, 10.0 mm of arginine were refluxed with 180 mm of sodium hydroxide solution until 11.5 mm of ammonia were evolved, yet no copper citrullinate could be separated. The possibility that this failure was due to the altered character of the reaction mixture is not to be excluded with finality. At any rate, successful hydrolysis of arginine to citrulline is readily accomplished by a 1:1 M ratio of sodium hydroxide to arginine.

The hydrolysis of arginine, as described in the experimental part, has been run in a single batch to give 65 gm. of citrulline; larger runs can probably be managed equally well. The operations are few and simple, and the yield, based on protein employed, is as good as that of any of the previous methods. A further important advantage is that arginine hydrochloride can be hydrolyzed and several gm. of free citrulline obtained in the same day.

The hydrolysis of arginine to citrulline is the reverse of their relationship in the urea scheme of Krebs and Henseleit (12). A closer analogy is to be found in the work of Bell (13), who obtained urea in good yield from the hydrolysis of guanidine with an equimolar quantity of base, whereas an excess of alkali was known to give carbon dioxide and ammonia (14). Bell found that free guanidine solutions were sufficiently alkaline of themselves to form urea on boiling. Attempts to obtain an analogous result with free arginine, in this work, failed to yield appreciable amounts of ammonia, as did boiling with an equimolar quantity of 0.1 N alkali. Normal alkali was, however, effective, and the more concentrated sodium hydroxide finally employed proved even more satisfactory.

EXPERIMENTAL

198 gm. of arginine hydrochloride (0.938 mole), prepared from U.S.P. gelatin by the method of Brand and Sandberg (15), were placed in a 1 liter round bottom flask with 330 ml. of 5.68 N sodium hydroxide (1.876 moles, 0.938 mole for neutralization of hydrochloride). The flask was fitted with a condenser and attached tube leading to slightly above the surface of a quantity of standard hydrochloric acid. The arginine solution, with glass

beads, was brought carefully to boiling, and refluxed $3\frac{1}{2}$ hours. At the end of this time, 0.64 mole of ammonia had been evolved. The cooled solution was acidified with glacial acetic acid and evaporated under reduced pressure on the hot water bath to approximately 250 ml., 300 ml. of water were added, and the evaporation was repeated. The residue was treated with 1500 ml. of absolute ethanol, and the liquid poured off after separation was complete. The crude citrulline was washed with further small quantities of alcohol and then dissolved in 800 ml. of water.

The solution was boiled 30 minutes with 79 gm. of copper oxide, and the copper citrullinate and unchanged copper oxide were filtered off.¹ The filtrate was evaporated down over a steam bath, and successive crops were removed by filtration. Four such crops were combined with the residue from the original filtration and the entire batch was washed thoroughly with water until the washings were colorless. A sample recrystallized from water decomposed at $257\text{--}258^\circ$ (corrected).² The copper salts were suspended in 1 liter of water, which was then saturated with hydrogen sulfide. The copper sulfide was coagulated by boiling, filtered hot, and the filtrate was cleared with norit. The solution was evaporated under reduced pressure on the hot water bath until crystals began to appear and was then treated with several volumes of absolute ethanol. The separated crystals were dried *in vacuo* over phosphorus pentoxide to give 65 gm. of white citrulline.

A sample was recrystallized from water and alcohol.

$C_6H_{13}N_3O_7$.	Calculated.	Amino N	7.99
	Found.	" "	7.94, 7.89 (microformol)

When examined in aqueous solution, and in dilute hydrochloric acid, the citrulline showed no optical activity.

SUMMARY

The theoretical hydrolysis of arginine to citrulline *in vitro* has been accomplished and discussed. The details for rapidly and

¹ In the smaller, pilot runs, no copper citrullinate crystallized at this stage, and it was possible to filter off copper oxide alone.

² The decomposition point of the copper salt is highly reproducible, whereas for the free citrulline values from $202\text{--}226^\circ$ are recorded in the literature, and ranges in the interval $200\text{--}218^\circ$ were found in this work.

conveniently preparing a 65 gm. quantity of citrulline with the aid of this single reaction have been presented.

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