XX. *l*-(+)-CITRULLINE

By ALLAN GODFREY GORNALL AND ANDREW HUNTER

From the Department of Pathological Chemistry, University of Toronto

(Received 30 December 1938)

CITRULLINE (α -amino- δ -carbamido-*n*-valeric acid) was first encountered as a constituent of the juice of the water-melon [Koga & Odaki, 1914; Wada, 1930]. Wada not only elucidated its constitution but produced it synthetically from ornithine. The substance was subsequently obtained by the action of putrefactive bacteria [Ackermann, 1931] and of *Bacillus pyocyaneus* [Horn, 1933] upon arginine, and also as a product of the tryptic digestion of casein [Wada, 1933]. More recently, methods have been described of preparing it in larger quantity from *dl*-ornithine [Kurtz, 1938] or from arginine [Fox, 1938].

Ackermann gives no information concerning the behaviour of his preparation towards polarized light, nor does Wada [1933] in the case of the product obtained from casein. All other specimens of citrulline hitherto prepared are described as optically inactive. The present paper describes for the first time the preparation of dextrorotatory citrulline and the determination of its specific rotatory power.¹

l-(+)-Citrulline was obtained simply by applying to *l*-(+)-ornithine the excellent method lately devised by Kurtz [1938] for the preparation of *dl*-citrulline from *dl*-ornithine. This method consists in condensing the copper salt of ornithine with urea to form copper citrullinate. We have used it successfully with *l*-(+)-ornithine hydrochloride prepared from gelatin by the enzymic method [Hunter, 1939].² An alternative procedure, when ornithine itself is not immediately available, is to apply Kurtz's method directly to the mixture of *l*-(+)-ornithine and urea obtained by hydrolysing *l*-(+)-arginine with arginase. For the success of this plan it is important that the hydrolysis of the arginine be complete, and that it be accomplished without introducing into the hydrolysate more than a minimum of extraneous material. The arginase solution used must therefore be at once purer and more potent than the ordinary crude glycerol extract of liver. One of us [Hunter, 1939] has recently described the preparation of a partly purified enzyme concentrate containing from 200 to 400 units [Hunter & Dauphinee, 1930] of arginase per ml. For the present purpose such a concentrate is entirely suitable.

The following account of one experiment among several shows the details of procedure adopted for the preparation of *l*-(+)-citrulline from *l*-(+)-arginine

¹ Since this paper was submitted for publication our attention has been drawn by M. Robert Duschinsky to his note (*C.R. Acad. Sci.* 1938, **207**, 735) in which are described the isolation of optically active citrulline from the water-melon and its synthesis by a method similar to our own. The figures given by M. Duschinsky for the rotation of the free amino-acid are in close agreement with those found by us.

² It is regretted that, in the discussion of the specific rotation of *l*-(+)-ornithine dihydrochloride (p. 35), reference was not made to the work of Lutz & Jirgensons (*Ber. dtsh. chem. Ges.* 1931, **66**, 1221). These authors pointed out the variation of the specific rotation of the salt with concentration; they found $[\alpha]_D^{20} + 9.7^\circ$ for $c = 1.01$ and $+ 13.3^\circ$ for $c = 4.1$.

hydrochloride. 10 g. of the latter salt were dissolved in 250 ml. of water, and enough *N* NaOH (3–4 ml.) was added to give a light pink colour with phenolphthalein. The solution was treated with concentrated arginase solution in such quantity as would supply 6000 units of the enzyme, and the mixture, adequately protected with toluene, was incubated at 37° for 18 hr.,¹ after which the Sakaguchi reaction for arginine was for all practical purposes negative. The mixture was then made acid to phenol red with acetic acid, boiled to coagulate proteins completely, filtered into a Claisen flask and evaporated *in vacuo* at 45–50° to about 50 ml. Frothing during the evaporation was controlled by addition of capryl alcohol.

The concentrated solution, containing equivalent quantities of ornithine hydrochloride and urea, was decolored with norite and filtered through a sintered glass filter. Flask and filter were washed with a few ml. of water, and the filtrate and washings brought, by dropwise addition of *N* NaOH, to *pH* just exceeding 7.0. The solution was then boiled, as described by Kurtz [1938], with 4 g. moist copper oxide, filtered, and, after addition of 10 g. urea, evaporated to 35 ml. After this concentrate, transferred to a tightly sealed pressure bottle, had been heated for 3 hr. in a boiling water bath, it yielded 7.4 g. of citrulline-copper (75% of the theoretical).

The poor solubility of this product made impossible any observation of its behaviour towards polarized light; but, since it yielded a dextrorotatory citrulline (see below), and was derived from *l*-(+)ornithine, it must have been *l*-(+)citrulline-copper. In colour, in crystalline form and in relative insolubility in water, it did not differ in any obvious way from *dl*-citrulline-copper, as described by Wada [1930] and Kurtz [1938]. Recrystallization, which is difficult, was not attempted, and the material was probably not entirely pure. (Found: N (Kjeldahl) 19.8; Cu 15.3%. $(C_6H_{12}O_3N_3)_2Cu$ requires N, 20.4; Cu, 15.44%.) The M.P. was 249°. For the purified racemic compound Wada and Ackermann each give M.P. 257–258°.

l-(+)Citrulline. 5 g. of the copper complex were suspended in 125 ml. of water and decomposed with H_2S . Coagulation of colloidal copper sulphide was effected by heating with norite, and the latter was separated by centrifuging. The supernatant, still slightly coloured, was warmed again with a little norite, filtered through retentive paper into a Claisen flask, evaporated *in vacuo* to about 10 ml., transferred to an Erlenmeyer flask and treated with sufficient alcohol to produce a slight opalescence. Crystallization was started by rubbing with a glass rod, and was allowed to progress until, in about 20 min., a loose mass of crystals filled the entire liquid. Thereafter further additions of alcohol were made, about 10 ml. at a time, until during about 2 hr. 100–150 ml. had been introduced. After a night in the ice-box the crystals were collected on a glass filter, washed four or five times with 5 ml. portions of chilled alcohol and dried, first at 95° and then *in vacuo* over P_2O_5 . The yield was 3.4 g., which is 80% of the theoretical; the overall yield from the original arginine was therefore 60%. (Found: N (Kjeldahl) 23.8%. After recrystallization 23.9%. $C_6H_{13}O_3N_3$ requires N, 23.99%.) M.P. 215–217°; after recrystallization, 219.5°. For *dl*-citrulline Wada [1933] gives 226°, Ackermann [1931] 220–222°, Kurtz [1938] 220–221°. In form the crystals were long thin prisms similar to those figured by Wada [1933].

The rotatory power of the product was measured (1) in pure aqueous solution and (2) in the presence of 1 mol. HCl. For the measurements in water

¹ Experience has shown that the enzyme quantities recently prescribed by one of us [Hunter, 1939] were unnecessarily large, and the time of action unnecessarily long.

we used three different preparations, A, B and C; for those with acid, preparation B and two recrystallized mixtures of various origin. All measurements were made at approximately 5 % concentration and at 21–23°. The results are collected in Table I.

Table I. *Specific rotation of l-(+)-citrulline*

Sample	Mols. HCl	<i>l</i>	<i>c</i>	α	$[\alpha]_D^{23^\circ}$ for $C_6H_{13}O_3N_3$	
A	0	2.2	5.005	+0.38°	+ 3.45°	} + 3.5°
B	0	2.2	5.025	+0.37°	+ 3.35°	
C	0	2.2	5.001	+0.40°	+ 3.64°	
B	1	2.2	5.016	+1.97°	+17.9°	} +17.9°
Recrystallized (1)	1	2.2	4.974	+1.96°	+17.9°	
Recrystallized (2)	1	2.2	5.009	+1.98°	+18.0°	

It will be seen that the observed difference between any two preparations hardly exceeded the experimental error, and that the rotatory power was not altered by recrystallization. There is, therefore, no reason to suppose that the material was other than pure *l*-(+)-citrulline. Its specific rotatory power in water is small—much smaller than that of the isomeric α -carbamido- δ -amino-valeric acid [Hunter, 1938]—but it is greatly increased by the presence of acid. In this respect citrulline resembles the related substances arginine and ornithine and indeed the amino-acids in general.

SUMMARY

A procedure is described in which the method of Kurtz is applied to the preparation, from *l*-(+)-ornithine or *l*-(+)-arginine, of *l*-(+)-citrulline. The yield from arginine is about 60 %. In 5 % aqueous solution the substance shows $[\alpha]_D + 3.5^\circ$, increasing, in the presence of 1 mol. HCl, to $+17.9^\circ$.

REFERENCES

- Ackermann (1931). *Hoppe-Seyl. Z.* **203**, 66.
- Fox (1938). *J. biol. Chem.* **123**, 687.
- Horn (1933). *Hoppe-Seyl. Z.* **216**, 244.
- Hunter (1938). *Biochem. J.* **32**, 826.
- (1939). *Biochem. J.* **33**, 27.
- & Dauphinee (1930). *J. biol. Chem.* **85**, 627.
- Koga & Odaki (1914). *J. Tokyo chem. Soc.* **35**, 519.
- Kurtz (1938). *J. biol. Chem.* **122**, 477.
- Wada (1930). *Biochem. Z.* **224**, 420.
- (1933). *Biochem. Z.* **257**, 1.