IV. ENZYMIC METHODS FOR THE PREPARATION OF ARGININE AND ORNITHINE

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(*Received 21 November 1938*)

THIS paper deals with two examples of the application of enzymes to preparative purposes: (1) the use of "trypsin" in the preparation of arginine, and (2) the use of arginase with urease in the preparation of ornithine. The first, while not devoid of practical utility, is described chiefly as a demonstration of the existence of substantial amounts of free arginine in pancreatic digests. The second is offered as a wholly satisfactory method for the preparation of ornithine, possessing certain advantages over any hitherto described.

1. *Isolation of arginine from pancreatic digests*

Some years ago Dauphinee & Hunter [1930] showed that digestion with commercial trypsin would split off, in a form accessible to the action of arginase, about 80% of the total potential arginine of certain proteins. The question, whether the arginase-susceptible material thus liberated is arginine itself or an arginine complex, was at that time left open. Later (unpublished) observations by Mr J. W. Chambers and the writer have indicated that it is probably a mixture, in which demonstrably free arginine forms at the beginning a relatively small, but, as proteolysis proceeds, an increasingly large, proportion of the whole. When, after about two weeks of digestion, that whole has reached its maximum, as much as 80 or 90% of it may be simple arginine.

This fact may be utilized in the preparation of arginine from protein by substituting for the usual acid hydrolysis the milder action of the pancreatic enzymes. The substitution has drawbacks, but also some advantages. Its drawbacks are (1) that, since the enzyme hydrolysis is incomplete, the yield of arginine is correspondingly reduced, and (2) that the need to separate the free arginine from arginine-containing complexes calls for a double precipitation with flavianic acid. The advantages are a simplification of the initial manipulations, and, more important, the avoidance of the partial racemization, which is produced by the long-continued action of boiling acids. The enzymic method leads, it will be shown, to a product of exceptional optical purity. How far this may compensate for the lower yield will depend upon circumstances.

The procedure is applicable to any protein which contains a relatively high proportion of arginine, and which at the same time is hydrolysable to a sufficient extent by the pancreatic enzymes. The detailed description which follows is adapted specifically to the case of gelatin.

250 g. air-dry gelatin are dissolved in 2500 ml. water at 37°. By appropriate addition of NaOH the solution is made definitely alkaline to phenolphthalein, and it is then treated with 10 g. of an active commercial trypsin suspended in about 50 ml. water. The mixture is covered with a layer of toluene and incubated at 37° for 15 to 20 days, i.e. until the "free" arginine, as determined by arginase, has reached a maximum.

The digest is now acidified with 5 *N* H_2SO_4 (at the rate of 2 ml. per 100), boiled, filtered from coagulated protein, heated almost to the boiling point, and treated with 450 ml. of a 20 % solution of flavianic acid. After a day or two in the ice-chest the granular yellow precipitate of crude arginine flavianate is collected on a large fritted glass filter (porosity 3), and washed with a cold 0·1 % solution of flavianic acid in 0·025 *N* H_2SO_4.

For purification the flavianate is suspended in about 1800 ml. water, and brought into solution with the minimum amount (about 20 ml.) of 5 *N* NaOH. The solution is diluted to about 2500 ml., heated again just short of boiling, and treated with a mixture of 50 ml. 5 *N* H_2SO_4 and 225 ml. (half the amount used in the first precipitation) of 20 % flavianic acid. In a minute or two the arginine flavianate begins to separate from the hot solution, this time in the form of large glistening iridescent orange-yellow leaflets. The mixture is allowed to cool slowly to room temperature, and is then set in the ice-chest for at least 24 hr. The precipitate is finally collected and washed in the same way as the first.

Precipitation with flavianic acid in the heat, as recommended, has several advantages. Not only does it yield the product in a form especially convenient for further manipulation, but it affords also a certain criterion of its purity; for if, under the conditions described, the flavianate assumes a granular form, it is almost certainly still contaminated.

From the purified arginine flavianate the arginine may be recovered by any of the standard methods, but the procedure adopted in this laboratory is the following. The flavianate is suspended in 500 ml. water and brought into solution with the minimum amount (about 12 ml.) of conc. NH_3. The solution is diluted to about 800 ml. and treated with a definite excess of cold saturated baryta water. The precipitate of barium flavianate is filtered off under suction, and washed thoroughly with very dilute baryta. From the mixed filtrate and washings the barium is precipitated quantitatively with H_2SO_4, and the residual flavianic acid is removed by treatment with the minimum amount of norite. $BaSO_4$ and norite are filtered off together. The colourless filtrate is freed of NH_3 by evaporation to a small volume, is then neutralized exactly with HCl and is finally evaporated to dryness, yielding directly a crystalline mass of arginine hydrochloride. This product, weighing as a rule between 15 and 17 g., is purified by dissolving it in a little water, rubbing up the solution with small amounts of alcohol until crystallization sets in, adding then about two more volumes of alcohol, and cooling overnight in the ice-chest.

In several experiments with this procedure the final yield of pure arginine hydrochloride has varied between 13·5 and 15·6 g. A representative sample gave by Kjeldahl 26·48 % N ($C_6H_{14}O_2N_4$.HCl requires 26·60 %), and left on incineration less than 0·1 % of ash. In the presence of seven extra molecules of HCl it had $[\alpha]_D^{23°} = 22·19°$ ($c = 11·47$; $l = 2·2$ dm.; $\alpha = +5·60°$). The highest value hitherto recorded for the specific rotatory power of arginine hydrochloride under the conditions specified is +21·95° [Hunter & Morrell, 1922; Hunter, 1929]. The product of the enzymic method of preparation was therefore *l*(+)-arginine of at least as great a degree of optical purity as has so far been attained. The experiments gave no indication whatever of the production of inactive arginine, such as has been described by Kutscher [1899; 1901] as occurring during the tryptic digestion of fibrin.

The gelatin used in the present work contained, according to the arginase method, 7·73 g. of potential arginine per 100 g. (air-dry). The yields of recrystallized hydrochloride mentioned above represent therefore only from 58 to 67 % of the possible maximum and roughly three-fourths of the amount recoverable

from a total hydrolysate by the excellent method of Cox [1928]. Of course, it has to be remembered that in the enzyme hydrolysates only 80 % or so of the total arginine had been so far liberated as to be accessible to the action of arginase [Dauphinee & Hunter, 1930]. Of this fraction the proportion finally accounted for as pure hydrochloride is from 73 to 84 %. This result was attained by procedures which, on the one hand, would be unlikely to bring about more than a minimal hydrolysis of arginine peptides, and which, on the other, certainly involved some losses. It seems therefore not unreasonable to conclude that in the final stages of pancreatic digestion at least 80 or possibly as much as 90 % of the arginase-susceptible material is actually free arginine.

The literature contains a few previous reports of the isolation of arginine in substance from pancreatic digests [Kossel & Mathews, 1898; Kutscher, 1898; 1899; 1901]; but the yields (never exactly stated) in these earlier experiments appear to have been relatively small.

2. *Preparation of ornithine*

The methods heretofore proposed for the preparation of ornithine from arginine have varied with respect to (1) the agent employed to hydrolyse the arginine, (2) the procedure followed in separating the ornithine from urea and from the hydrolysing reagent and (3) the form in which the ornithine has been finally isolated. For the hydrolysis use has been made of $Ba(OH)_2$ [Schulze & Winterstein, 1898; Bergmann & Zervas, 1926; Kurtz, 1938], NaOH [Boon & Robson, 1935] and arginase [Kiesel, 1911; 1922; Felix & Röthler, 1925; Vickery & Cook, 1931]. To separate the ornithine Schulze & Winterstein [1898] employed benzoylation, Bergmann & Zervas [1926] and Boon & Robson [1935] condensation with salicylaldehyde, and the other authors (Kurtz excepted) precipitation with phosphotungstic acid. As a rule the base has ultimately been converted into the hydrochloride; but Kiesel [1911] isolated it as carbonate, Felix & Röthler [1925] and Vickery & Cook [1931] as picrate, Kurtz [1938] as sulphate.

The earlier methods, whatever combination of procedures they represented, gave very unsatisfactory yields. Schulze & Winterstein [1898] appear to have obtained from arginine at most 30 % of the theoretical amount of ornithine; and no better result was claimed for any of the alternative procedures published prior to 1931. Parenthetically it may be mentioned that in 1929 Mr H. B. Collier, working under the writer's direction, obtained ornithine hydrochloride in yields up to 45 % by the action of arginase upon arginine, followed by condensation of the product with salicylaldehyde in the presence of $Ba(OH)_2$. Collier's results were left unpublished because of uncontrollable irregularities encountered in the application of the salicylaldehyde method. Vickery & Cook [1931] also had difficulties with this method, but by the use, under special conditions, of phosphotungstic acid obtained from an arginase digest ornithine (as dipicrate) corresponding to 55 % of the arginine taken.

Really satisfying yields ("approximately theoretical") were first attained by Boon & Robson [1935], who recommended hydrolysis (of carbamido-arginine) with 20 % NaOH, separation of ornithine as the barium salicylidene derivative, and conversion of the latter into the hydrochloride. In the yet more recent method of Kurtz [1938] carbamido-arginine is treated with baryta under conditions which not only split it into ornithine and urea, but which also ensure the destruction of the latter. Since the baryta itself is readily removed, preliminary separation of the ornithine can be dispensed with, and it is precipitated directly (as sulphate) by alcohol. Yields range from 76 to 82 %.

The method of Kurtz is the simplest yet proposed; but, like all methods depending on prolonged hydrolysis by alkalis, it has the drawback of yielding a product which is either partially or completely racemized. If the natural dextrorotatory ornithine is required, it is desirable, if not imperative, to resort in the first place to enzymic hydrolysis, and to restrict to the necessary minimum any subsequent exposure of the product to the action of alkali. Now for the successful application of the enzymic method two points are of practical importance. In the first place, in order to effect a complete hydrolysis of the arginine (which previous enzyme methods have evidently failed to do) it is necessary to use a large amount of arginase—about 1000 units [Hunter & Dauphinee, 1930] per g. arginine. In the second, it is obviously desirable to introduce along with the enzyme as little extraneous and inert material as possible. From this point of view a crude glycerol extract of liver is a quite unsuitable reagent. Later there is described the preparation from aqueous liver extracts of a concentrated arginase solution containing in 1 ml. from 250 to 380 units of the enzyme but only a fraction of the solid material with which that enzyme was originally associated. Under appropriate conditions from 2·5 to 4 ml. of such a solution amply suffice for the complete hydrolysis of 1 g. arginine, yet introduce little that cannot afterwards be removed by heat coagulation. There is therefore a possibility of preparing digests, which contain hardly anything in solution but an ornithine salt and urea.

It was thought at first that the separation of these two substances from one another might be accomplished in a very simple way by taking advantage of their greatly differing solubilities in alcohol. Had this proved possible, the steps necessary for the isolation of ornithine would have been few and straightforward. Unfortunately attempts to precipitate ornithine from the concentrated protein-free digest with alcohol, or to recrystallize from alcohol the solid residue left on evaporation, gave disappointingly small yields of a product which was always contaminated with urea. It remained necessary, therefore, either (*a*) to precipitate the ornithine first as phosphotungstate or other insoluble compound or (*b*) to destroy the urea. Since one of the objects sought was the elimination of the usual intermediate precipitation, the alternative adopted was the second; and in order to keep the whole method an enzymic one the agent chosen for the destruction of urea was urease. This can be quite conveniently applied in combination with arginase, so that the decomposition of urea may proceed *pari passu* with the production of ornithine. The alkaline reaction rapidly produced by the development of ammonium carbonate favours the action of the arginase without unduly depressing that of the urease. When the process is complete, both the ammonium and the carbonate ions can be got rid of by distilling the mixture with an excess of baryta. The barium having been in its turn removed, one is left with a solution of ornithine (or an ornithine salt), which can be freed from residual (mostly inorganic) impurities in the ordinary way.

The methods of preparation described below are based on the principles thus outlined.

(A) *Enzyme reagents required*

Arginase. A crude arginase extract is prepared according to the method of Hunter & Dauphinee [1930] with the single change that water is substituted for 75% glycerol. One volume of this extract is mixed with 1·2 volumes of acetone. The flocculent precipitate is separated by centrifuging, and extracted with half the original vol. of water. Any insoluble material is filtered off, and the filtrate is treated again with 1·2 vol. of acetone. The second precipitate is taken up in

one-fourth of the original volume, and to this final solution cobalt chloride is added in the proportion of one drop of a 1 % solution for each 10 ml. The activity of the solution is determined by the method of Hunter & Dauphinee [1930]. It should contain the least 200, preferably between 300 and 400, units of arginase per ml.

Urease. 10 g. jack-bean flour are shaken for 10 min. with 100 ml. of 30 % alcohol, and the mixture is then filtered.

(B) *Preparation of ornithine monohydrochloride from arginine hydrochloride*

To a solution of 10 g. arginine hydrochloride in 250 ml. water there are added (1) the prescribed arginase solution in amount sufficient to provide 8000 units of the enzyme, and (2) 10 ml. of the urease solution. The mixture, protected by toluene, is incubated at 37°. At first faintly acid, it rapidly develops an alkaline reaction and a strongly ammoniacal odour. After 4 or 5 days it is made just acid to Congo red with 5 *N* H_2SO_4 (about 18 ml.), and is boiled to coagulate proteins. (Acidification may, if it is thought worth while, be preceded by a preliminary distillation *in vacuo* with alcohol; this effects the removal of a considerable proportion of the NH_3, and reduces correspondingly the subsequent consumption of H_2SO_4 and baryta.) The mixture is cooled, and treated with a considerable excess (about 225 ml.) of saturated baryta water. The precipitate of coagulated protein and insoluble Ba salts is filtered off and washed with dilute baryta water. Filtrate and washings are transferred to a large Claisen flask, and freed from NH_3 by distillation *in vacuo* at a temperature not exceeding 50°. Frothing, when it occurs, is controlled by liberal additions of alcohol, repeated, as often as may be necessary, until the evolution of NH_3 comes to an end. The distillation residue should then be negative to Nessler's reagent. If it is not, more baryta is added, and the distillation continued.

When one is certain that the ammonia has been completely removed, the residue is freed exactly from barium by H_2SO_4, and filtered. It should now be neutral or only slightly alkaline, and give no more than a feeble Sakaguchi reaction for arginine. Having, if necessary, been made exactly neutral with HCl, it is concentrated on the water bath to about 75 ml., decolorized by heating with a generous addition of norite, filtered, and evaporated to dryness. If the product becomes seriously discoloured during evaporation, the treatment with norite is repeated. There is thus obtained a crude ornithine monohydrochloride, nearly white, but contaminated as a rule with a considerable quantity of inorganic salts.

For its purification the crude material is dissolved, by prolonged boiling under a reflux condenser, in the smallest possible volume of 75 % alcohol. If necessary, the hot solution is rapidly filtered through a fritted glass filter. Crystallization begins almost immediately. After a day in the cold chamber the crystals are collected, and washed with a little 95 % alcohol. This first crop should weigh about 6 g., and should not contain more than 0·3 % ash. A second crop of equal purity is obtained by evaporating the mother liquors to dryness and again recrystallizing the residue from 75 % alcohol. In this way the yield may be increased to 7 g. or more.

The results of two experiments with this method, including the N (Kjeldahl) and ash contents of the products, are given in Table I. The lower yield of the first experiment is accounted for by the fact that in this, an early one, only one crop of crystals was collected. With a little experience it is easy to reproduce the improved results of the second.

(C) *Preparation of ornithine hydrochloride or sulphate from protein*

The success of the enzymic method as applied to arginine itself suggested that it might be possible by similar means to isolate ornithine directly from any protein hydrolysate reasonably rich in arginine. In this way one would not need first to prepare (or procure) pure arginine. This idea, in its first shape, was frustrated by the difficulty of separating the ornithine from the many other substances in the arginase-treated hydrolysate. In a modified form it proved to be entirely feasible. Neither arginase nor urease is inactivated by flavianic acid. All that is necessary therefore to obtain ornithine from a protein is to hydrolyse the latter, precipitate the liberated arginine once with flavianic acid, and treat the arginine flavianate in the same way as one would the hydrochloride. When this is done, the baryta used to liberate NH_3 serves also to remove the bulk of the flavianic acid. The ornithine may be isolated either as hydrochloride or as sulphate. In detail the procedure is as follows:

A convenient weight of some appropriate protein (say 200 g. gelatin or 50 g. protamine) is hydrolysed by boiling for 12 hr. with a ten-fold quantity of 20 % HCl. The hydrolysate is freed, as far as may be possible, from HCl by concentration *in vacuo* to a thick syrup. It is then taken up in water, neutralized to Congo-red with 40 % NaOH, decolorized with norite, filtered, and diluted to approximately its original volume. If its estimated concentration of arginine is then much greater than 1 % (as would be the case with a protamine hydrolysate) it may with advantage be diluted even further. The solution is now heated to the point of boiling, and treated with 20 % flavianic acid. The quantity of this to be used is calculated at the rate of 20 ml. for each g. of expected arginine. The mixture having been cooled, first to room temperature and then for at least 24 hr. in the ice-chest, the flavianate is collected under suction on a large fritted-glass funnel, washed, suspended in several vol. of water and brought into solution with the minimum amount of NH_3. The solution is diluted until it contains approximately 3 % of arginine, and is then digested with arginase and urease. The quantities of these are calculated so as to supply 1000 units of arginase and 1·4 ml. of urease solution for each g. of arginine believed to be present. Digestion is continued, in the presence of toluene, for 4 days at 37°.

With or without a preliminary vacuum distillation (see Section B) the strongly ammoniacal digest is neutralized with 5 *N* H_2SO_4 and boiled. Additional acid is added sufficient to ensure the exact coagulation of the proteins. The cooled mixture is treated with an adequate excess of saturated baryta water, and filtered. The precipitate is washed with dilute baryta. Filtrate and washings are combined, and freed from NH_3 by vacuum distillation just as in the preparation from arginine.

The further treatment of the material depends upon whether it is decided to isolate the ornithine as sulphate or as hydrochloride.

(*a*) *Ornithine monohydrochloride.* To obtain the hydrochloride the solution left in the distilling flask is filtered (if necessary), exactly freed from Ba with H_2SO_4, shaken with enough norite to remove residual flavianic acid, filtered, neutralized to litmus with HCl, and concentrated *in vacuo* to about 100 ml. The colour which usually develops during this concentration is removed by boiling with norite, and the filtered solution is evaporated to dryness. The crude hydrochloride is finally recrystallized from 75 % alcohol in the manner described already in Section B.

(*b*) *Ornithine monosulphate.* To obtain the sulphate the solution is neutralized exactly to litmus with H_2SO_4, a step which incidentally frees it from Ba. It is

then decolorized, filtered, concentrated, again decolorized—all in the same way as with the hydrochloride—and finally evaporated on the water bath, not to dryness, but only until crystallization begins. When it has cooled, it is stirred vigorously with successive small additions of absolute alcohol. This brings about the separation of more sulphate, at first in the form of milky globules, presently coalescing into a heavy oil. As the operation is continued, the oil gradually solidifies, the crystals get harder and harder, and finally the whole is converted into a heavy crystalline powder. More alcohol is added, and the mixture is left overnight in the ice-box. The alcohol is then poured off, and the crystals are washed, by stirring and decantation, 3 times with cold 95 % alcohol. The crystals are allowed to dry in the air, being stirred frequently the while, in order to prevent them from forming heavy crusts or sticking to the sides of the dish. They are then dried further in the desiccator and finally at 110°.

It will be gathered from this prescription that the physical properties of the sulphate differ from those of the hydrochloride. Kossel & Weiss [1909] describe an optically inactive ornithine sulphate which can be readily recrystallized from hot 60 % alcohol. The enzymically prepared sulphate, which is dextrorotatory, cannot be conveniently recrystallized in this way. Heated with either 60 or 75 % alcohol it yields for the most part only an oil. Recrystallization from 85 % alcohol is possible, but requires an impracticably large volume of solvent. Collected and dried on a filter in the usual way, the crystals form a hard cement-like cake, which can hardly be detached or broken up even with a steel spatula. By the methods of crystallization, washing and drying described above one escapes these several inconveniences.

The preparation of the sulphate involves fewer and simpler manipulations than that of the hydrochloride, and its yield is at least equally good. Its sole disadvantage is that the product may contain a rather high proportion—up to 3·6 % of ash. This consists mainly of calcium sulphate. It can be eliminated by repeated recrystallization from water, but the ornithine salt itself is so soluble, that purification by such means would be highly unprofitable.

The methods described have been applied with success to gelatin and to protamine, and Table I gives the data of three experiments with these materials. Two (with gelatin) exemplify the preparation of ornithine sulphate, the third (with protamine) that of the hydrochloride. The gelatin used was of the same brand as that employed in Part I for the preparation of arginine, so that 100 g. (air-dry) were capable of yielding 7·48 g. of ornithine monohydrochloride or 8·04 g. of ornithine monosulphate. The protamine was derived (as a sulphate) from an unidentified species of Pacific Coast salmon (*Oncorhynchus*). Its degree of purity was unknown. Analysis showed that it contained 11·9 % water, 18·5 % H_2SO_4 and 19·9 % N. On ignition it left 5·54 % ash, largely sulphate. The arginase method indicated that arginine accounted for 77·2 % of the total N—much less than the 89·3 % found by Waldschmidt-Leitz *et al.* [1931] for purified salmine. According to these data 100 g. of the air-dry sulphate contained 47·75 g. of potential arginine. The equivalent amount of ornithine monohydrochloride is 46·2 g. The high arginine content of protamine makes it a particularly remunerative starting material for the preparation of ornithine.

On a review of the figures in Table I it may be seen that, whether one starts from arginine itself or from protein, the enzymic method is capable of yielding from 80 to 90 % of the theoretical amount of ornithine. With respect to nitrogen content the product, whether in the form of sulphate or of hydrochloride, shows a high degree of purity, and only the sulphate contains more than a trace of inorganic impurity.

Table I

	Arginine hydrochloride		Gelatin		Protamine sulphate
Weight taken, air-dry (g.)	—		200		50
Weight taken, ash- and water-free (g.)	10·00		175·6		41·3
Arginine content (g.)	8·27		15·5		23·9
Ornithine monohydrochloride corresponding (g.)	8·00		—		23·1
Ornithine monosulphate corresponding (g.)	—		16·1		—
	(1)	(2)	(1)	(2)	
Ornithine monohydrochloride recovered (g.)	6·00	7·17	—	—	18·7
Ornithine monosulphate* recovered (g.)	—	—	14·4	14·0	—
Percentage yield*	75	90	90	87	81
Ash, % of product	0·28	0·10	3·63	3·38	0·04
Nitrogen,* % of product	16·42	16·48	15·47	15·20	16·52
Nitrogen, % of product, theoretical	16·62		15·47		16·62

* On an ash-free basis.

Determinations of specific rotatory power were made (1) on the monosulphate No. 1 from gelatin, (2) on the monohydrochloride from protamine, (3) on the dihydrochloride, prepared by adding the calculated amount of HCl to a solution of the monohydrochloride. The details and results of the measurements made are given in Table II. It should be stated that the first measurement on the monohydrochloride was made before, the others after, a single recrystallization from alcohol. This recrystallization, it will be seen, did not increase the specific rotation. The first and second measurements reported on the sulphate were made after, respectively, one and two recrystallizations from water. The product of the first recrystallization still retained 0·90 % ash; that of the second had only 0·16 %. The specific rotations of the two are not significantly different. The original sulphate, with 3·6 % ash, gave a value rather lower than that shown in Table II. Whether this should be attributed to a racemic admixture or to other impurities is uncertain.

Table II. *Specific rotations of ornithine salts*

					$[\alpha]_D$ calculated	
Salt of ornithine	t	l	c	α	For the salt	For ornithine
Monohydrochloride	23°	2·2	22·37	+4·98°	+10·13° } +10·08°	+12·85°
,,	23	2·2	21·99	+4·85	+10·03	
,,	23	2·2	5·496	+1·33	+11·00 } +11·0	+14·0
,,	25	2·2	5·693	+1·37	+10·94	
Dihydrochloride	23	2·2	13·37	+5·11	+17·36	+26·96
,,	25	2·2	10·60	+4·01	+17·20	+26·71
,,	23	2·2	5·300	+1·95	+16·7	+25·9
Monosulphate	22	2·2	6·070*	+1·15	+ 8·61 } + 8·7	+11·9
,,	22	2·2	5·864*	+1·13	+ 8·76	

* On an ash-free basis

As far as the mono-salts of ornithine are concerned, the only previous determinations of optical activity are that of Vickery & Cook [1931], who found for the enzymically prepared monosulphate $[\alpha]_D^{25°} = +8·4°$, and that of Dirr & Späth [1935], who give for the monohydrochloride $[\alpha]_D^{20°} = +12·12$. For the first of these salts the values here exhibited are appreciably higher, for the second quite definitely lower. The discrepancies may be more apparent than real, for neither Dirr & Späth nor Vickery & Cook have recorded concentrations or other relevant details. In any case the lower values now assigned to the monohydrochloride are supported by the results for the dihydrochloride, into which it was

converted. For this salt there are on record three earlier determinations—that of Schulze & Winterstein [1901], who found $[\alpha]_D = +16{\cdot}8°$ ($c = 5{\cdot}06$; $t = 10°$), that of Kossel & Dakin [1904], who give $+16{\cdot}6°$ (no details), and that of Bergmann & Zervas [1926], who give $+16{\cdot}5°$ ($c = 4{\cdot}72$; $t = 19°$). With these figures the present value $+16{\cdot}7°$ (for $c = 5{\cdot}30$ and $t = 23°$) is in satisfactory agreement. As far, therefore, as the data enable one to judge, the enzymically prepared material was pure $l(+)$-ornithine hydrochloride.

The specific rotatory power of free ornithine is given by Vickery & Cook (1931) as $+11{\cdot}5°$. From the figures in Table II it may be seen that the rotation increases as the base combines with increasing proportions of acid, and that the effect of HCl in this respect is greater than that of H_2SO_4. It is further evident that both for the mono- and the di-hydrochloride specific rotatory power varies more than a little with concentration. In all these respects the behaviour of ornithine shows, quantitatively as well as qualitatively, a striking resemblance to that of arginine [Gulewitsch, 1899].

Summary

Methods are described which exemplify (1) the use of "trypsin" in the preparation of arginine (as hydrochloride) from protein, and (2) the combined use of arginase and urease in the preparation of ornithine (as hydrochloride or sulphate) from arginine or from protein. The methods for ornithine give yields up to 90% of the theoretical. The products are shown to possess a high degree of optical purity. The specific rotatory power of $l(+)$-ornithine monosulphate (in 6% concentration) is $+8{\cdot}7°$, while that of the monohydrochloride varies from $+10{\cdot}08°$ (at 22%) to $+11{\cdot}0°$ (at 5·5%), and that of the dihydrochloride from $+17{\cdot}36°$ (at 13%) to $+16{\cdot}7°$ (at 5%).

In the development of the method for arginine the writer had the technical assistance of Mr C. H. Downs, and at all points of the investigation that of Mr C. E. Downs. The polarimetric observations were made by Mr A. G. Gornall. The protamine used was furnished by the Connaught Laboratories of the University of Toronto. To all these helpers the writer gratefully acknowledges his indebtedness.

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