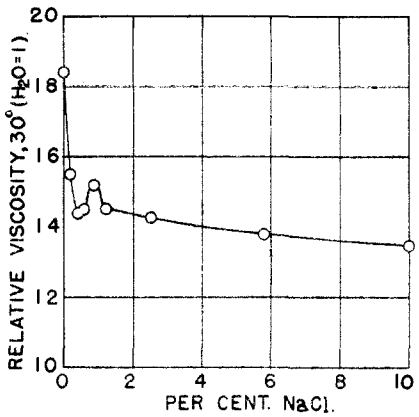


Fig. 2.—Relative viscosity of 0.15% sodium desoxyribonucleate in sodium chloride solution, as a function of sodium chloride concentration.

prepared in dilute citric acid at *p*H 4.0,[14] one gram of nuclei furnishes a convenient amount of starting material. The method can also be applied to a single rat liver, although when working with such a small amount of material it is difficult to obtain as proportionately high a yield of product as when one uses 50 g. of calf thymus as described in the experimental procedure. There seems to be no reason why the method could not be adapted to large scale work, if desired, provided of course that volumes of solutions and amounts of reagents added are adjusted to the amount of material being processed.

It is believed that this work also furnishes further experimental evidence supporting the concept that desoxyribonucleic acid is firmly bound in cell nuclei in the natural state, and becomes soluble only after autolysis or protein denaturation.[15]

(14) A. L. Dounce, *J. Biol. Chem.*, **151**, 221 (1943).

(15) A. L. Dounce, Chapter 5, Vol. I, Part I in "The Enzymes," edited by J. B. Sumner and K. Myrbäck, Academic Press, Inc., New York, N. Y., 1950.

ROCHESTER, N. Y. RECEIVED SEPTEMBER 19, 1951

[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH; AND THE DEPARTMENT OF CHEMISTRY, TEMPLE UNIVERSITY]

The Biosynthesis of Arginine by *Torulopsis Utilis*[1]

BY MURRAY STRASSMAN[2] AND SIDNEY WEINHOUSE

To obtain information concerning the biosynthesis of arginine in yeast, this amino acid was isolated from cell material grown on glucose in the presence of various small molecules labeled with C^{14}. The ornithine moiety of arginine synthesized in the presence of methyl- and carboxyl-labeled acetate, methylene- and carboxyl-labeled glycine, carboxyl-labeled lactate, and formate had the distribution of labeled carbon which would be expected in α-ketoglutarate formed during the intermediary metabolism of these labeled substances *via* the citric acid cycle. It was therefore concluded, in confirmation of earlier studies, that the intact α-ketoglutarate carbon skeleton is the direct source of the 5-carbon chain of arginine, presumably *via* glutamate, ornithine and citrulline.

As part of a study of metabolic processes in fungi the biosynthesis of various cell components is being investigated. Intensive study in recent years of the metabolism of such substances as CO_2, formate, acetate, glycine, etc., by the isotopic tracer method has elucidated many of the metabolic routes of these substances. As a result it is often possible by determining the distribution of C^{14} among the carbon atoms of a particular cell component produced during growth of an organism on labeled simple substrates, to trace the biosynthetic pathway of the substance in question. In the present study information on the mechanism of arginine synthesis in *Torulopsis utilis* was obtained in this fashion.

Previous work has indicated that the carbon chain of arginine probably arises from glutamic acid. Evidence for a close relationship of glutamate, proline and ornithine in rats has been provided by studies of Roloff, Ratner and Schoenheimer[3] by means of deuterium-labeling experiments, and further information on the interrelationships of these three amino acids has been provided by studies of Stetten and Schoenheimer[4] with N^{15}-labeled proline. Evidence for similar pathways for arginine synthesis in *Penicillium* and in *E. Coli* has also been provided, since mutant strains of these organisms have been found in which arginine synthesis may be blocked at any one of five places: glutamic acid, proline, ornithine, citrulline or arginine.[5,6]

In the present study the yeast was cultivated on glucose as essentially the only carbon source, together with tracer quantities of the following labeled compounds: methyl- and carboxyl-labeled acetate, methylene- and carboxyl-labeled glycine, carboxyl-labeled lactate and formate. The arginines obtained on hydrolysis of the harvested and washed cells were submitted to chemical degradation to ascertain the distribution of radioactivity among the six arginine carbons. Although the degradation procedure employed did not distinguish each carbon uniquely, it was sufficiently detailed to demonstrate rather convincingly that the ornithine moiety of arginine arises directly from the α-ketoglutarate carbon skeleton.

Experimental Results

The yeast used in these experiments was a strain of *Torulopsis utilis* obtained from the Fleischmann Laboratories.

(1) This work was done under contract with the Atomic Energy Commission (Contract No. AT(30-1)777) and aided by an institutional grant from the American Cancer Society to the Institute for Cancer Research.

(2) This work will be included in a thesis to be submitted by Murray Strassman to the Graduate School of Temple University in partial fulfillment of the requirements for the Ph.D. degree.

(3) M. Roloff, S. Ratner and R. Schoenheimer, *J. Biol. Chem.*, **136**, 561 (1940).

(4) M. R. Stetten and R. Schoenheimer, *ibid.*, **153**, 113 (1944).

(5) D. Bonner, *Am. J. Botany*, **33**, 788 (1946).

(6) J. Lederberg and E. L. Tatum, *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 113 (1946).

Details of the procedures employed in the cultivation of this organism are given in the previous publication.[7] In each experiment the labeled substance, in the form of the sodium salt, was added in an amount of 0.3 millimole, containing a total activity of 10 microcuries. After 2 or 3 days growth at room temperature, at which time 6 to 8 g. of cells (dry weight) were formed, the yeast was harvested by centrifugation, washed repeatedly with water, and the lipides removed by extraction with successive portions of alcohol and ether in a soxhlet apparatus. The lipide-free yeast was hydrolyzed by 16 hours reflux in 75 ml. of 6 *M* HCl solution. The hydrolysate was filtered from humin and evaporated to dryness under reduced pressure. The residue was taken up in 25-ml. of water and the amino acids freed from carbohydrate and other substances by precipitation with mercuric acetate according to the procedure of Neuberg.[8,9] The mercuric salts were separated by centrifugation and washing with 80% ethanol, and the free amino acids were regenerated by decomposition of the mercury salts with H_2S. After removal of the tyrosine by crystallization[10] and precipitation of the dicarboxylic acids as their barium salts, arginine was isolated as the flavianate according to the method of Block and Bolling.[9] The flavianate was decomposed by dissolving in 30–40 ml. of 2 *M* HCl and the flavianic acid removed by extraction with three 15-ml. portions of butanol. The aqueous solution was treated with decolorizing charcoal, evaporated to dryness under reduced pressure, and the residue was taken up in 0.8 to 1.0 ml. of absolute ethanol. On addition of a few drops of aniline, arginine monohydrochloride separated, and was collected by filtration after allowing crystallization to proceed to completion in the refrigerator overnight. Yields of arginine monohydrochloride ranged from 70–100 mg. in the various experiments. The purity of each sample was established by paper chromatography, and in no case were significant quantities of other amino acids found.

The activities of the arginines obtained are given in Table I. The most efficient precursors of arginine carbon appeared to be the α- and carboxyl-carbons of acetate and the α-carbon of glycine, the corresponding arginines having over-all specific activities ranging from 13,000 to 17,000 c./min. The carboxyls of lactate and glycine were incorporated to a much reduced extent, and formate carbon displayed the lowest incorporation of all carbons tested.

TABLE I

INCORPORATION OF CARBON FROM LABELED SUBSTRATES IN ARGININE SYNTHESIZED BY *Torulopsis Utilis*

Substrate	Position of label	Activity of arginine[a]
Acetate	Carboxyl	17,160
Acetate	Methyl	15,120
Glycine	Methylene	13,400
Lactate	Carboxyl	4,540
Glycine	Carboxyl	2,800
Formate	.....	1,202

[a] Activities of arginine monohydrochloride in counts per minute per standard 7.5 sq. cm. dish at "infinite thickness."

Degradation of Arginine: Removal of Guanido Carbon.—Portions of the original samples were diluted with non-isotopic carrier arginine monohydrochloride to give samples of sufficient quantity (0.6 to 1.0 mM.) and activity (600–1500 c./min.) for the degradation. The diluted material was recrystallized from ethanol to a constant activity, which in all cases corresponded closely to the value calculated from the original activity. Approximately 1 mM. of the diluted salt was dissolved in 10 ml. of water in a 100-ml. 3-neck flask carrying a dropping funnel, a lead-in tube extending to the bottom, and a condenser surmounted with a tube leading to an absorption tower filled with glass beads. Six ml. of 50% CO_2-free NaOH was added and the flask was heated in an oil-bath for 6–7 hours at 140°. Ten ml. of an 0.5 *M* CO_2-free sodium hydroxide solution was placed in the bead-tower, and, while a moderate stream of CO_2-free air was being drawn through the flask and bead-tower, sufficient 1:10 sulfuric acid was added for acidification. After bringing the solution to boiling, 20 minutes were allowed to complete absorption of CO_2, after which the contents of the bead-tower were washed down and the carbonate precipitated by addition of $BaCl_2$. This method was found to give quantitative recovery of the arginine guanido carbon.

Oxidation of Ornithine to Succinic Acid and CO_2.—Numerous exploratory experiments with ornithine resulted in the following procedure which gives a quantitative yield of CO_2 (1 mole per mole of ornithine). Though the recoveries of succinic acid in experiments on a 0.5 to 1.0 mM. scale were only about 30–40%, it seemed fairly certain that the reaction involved was that shown in equation I

$$\overset{5\ 4\ 3\ 2\ \ \ 1}{H_2NCH_2CH_2CH_2CHNH_2—COOH} + O_2 \longrightarrow \overset{5\ 4\ 3\ 2\ \ \ 1}{HOOC—CH_2CH_2COOH} + CO_2 + NH_3 \quad (I)$$

and it is therefore assumed that the CO_2 represents the carboxyl carbon of the ornithine moiety and that the succinate represents the remaining 4 carbons.

The acidified solution of ornithine, from which the arginine guanido carbon was removed, was cooled, washed into a 200-ml. 3-necked flask, diluted to 120 ml., and with a set-up similar to the previous step, the flask was heated in a boiling water-bath while sufficient 1.5 *N* potassium permanganate solution (8–10 ml.) was added to give a permanent pink color. The CO_2 evolved was collected in a bead-tower by means of an air-stream and precipitated with barium chloride.

Isolation of Succinic Acid.—The remaining solution, after cooling, was extracted continuously with ethyl ether for 14 to 18 hours. The extract was concentrated, taken up in 15 ml. of water, neutralized to a brom cresol blue end-point with ammonia, and the silver salt precipitated by addition of 5 ml. of 10% silver nitrate. The silver succinate was decomposed by suspending in water and passing H_2S into the heated suspension; and pure succinic acid was isolated by filtration and evaporation of the resulting clear solution. The crystalline product thus obtained invariably had the correct melting point for succinic acid. If necessary this product was diluted with carrier succinic acid in order to obtain sufficient material for the subsequent pyrolysis.

Pyrolysis of Barium Succinate.—The procedure employed for this step has been described previously by Kushner and Weinhouse.[11] The carboxyl carbons were assayed directly by liberation of the CO_2 from the pyrolysate by the addition of acid; values for the methylene carbons were calculated by difference between the activity of the carboxyl carbons and the over-all activity of the four succinate carbons.

Counting Procedures.—All samples were counted in a flow-gas counter. Radioactivities are expressed as counts per minute per standard dish of 7.6 sq. cm. area, corrected when necessary for self-absorption to a layer of "infinite thickness." Radioactive substrates were obtained either from Tracerlab, Inc., or from the Isotopes Division of the U. S. Atomic Energy Commission on allocation by the latter.

Distribution of Activity among Arginine Carbons.—To present data from different experiments in a uniform manner, the specific activity values for individual or groups of carbon atoms are given in percentages of the over-all specific activity of all of the arginine carbons. In Table II there are given complete data for acetate methyl and carboxyl, glycine methylene and lactate carboxyl carbons.

(7) S. Weinhouse, R. H. Millington and M. Strassman, THIS JOURNAL, **73**, 1421 (1951).

(8) C. Neuberg and J. Kerb, *Biochem. Z.*, **40**, 498 (1912).

(9) R. J. Block and D. Bolling, "Amino Acid Composition of Proteins and Foods," C. C. Thomas, Springfield, 1945, p. 287.

(10) None of the experiments described herein yielded tyrosines with any appreciable activity. Baddiley, *et al.* (*J. Biol. Chem.*, **183**, 771 (1950)) reported activities from the methyl and carboxyl carbons of acetate to be present in various positions of the tyrosine ring and side-chain; however, these experiments were carried out with yeast grown on acetate as the main carbon source. In experiments similar to those reported here, Gilvarg and Bloch (THIS JOURNAL, **72**, 5791 (1950)) also found that acetate was not appreciably incorporated in tyrosine but that glucose carbon is so utilized, presumably by a process of direct cyclization of the intact 6-carbon chain. Gilvarg and Bloch have pointed out that in the experiments of Baddiley, *et al.*, acetate was probably utilized indirectly for tyrosine synthesis only insofar as it was converted to glucose.

(11) M. Kushner and S. Weinhouse, THIS JOURNAL, **71**, 3558 (1949).

Inasmuch as the arginines synthesized in presence of carboxyl-labeled glycine and formate had essentially all of their activity in the guanido and carboxyl carbons, further degradation of these samples was not undertaken. In designating the individual arginine carbons numbering was begun at the carboxyl, and the guanido carbon is number six.

TABLE II

DISTRIBUTION OF LABELED CARBON AMONG ARGININE CARBONS

Individual values are specific activities based on a value of 100 for over-all activity of the six arginine carbons. All samples were counted as $BaCO_3$

Precursor	Position of label	Carbon atom of arginine 1	6	2–5	2,5	3,4
Acetate	Carboxyl	167	52	71	152	−10
Acetate	Methyl	75	26	118	73	163
Glycine	Methylene	155	41	108	127	90
Glycine	Carboxyl	118	370	~0	...	..
Lactate	Carboxyl	102	364	10	...	..
Formate	.....	100	441	~0	...	..

The data in Table II show that acetate and glycine methylene activity is present in relatively high concentration in the arginine carbon chain, with rather low activity in the number six (guanido) carbon. From the work on urea synthesis[12,13] and the studies of Delluva and Wilson[14] in rats, the guanido carbon can be presumed to have its origin in CO_2. Since this carbon in the arginines was rather low in comparison with the activity of the carbon chain, it can be concluded that both acetate carbons and the glycine α-carbon are directly incorporated in the arginine carbon chain (the ornithine moiety). By contrast, lactate carboxyl, glycine carboxyl and formate activity appeared preponderantly in the guanido carbon, indicating that the main pathway of incorporation of these carbons is indirect, *viz.*, by CO_2-fixation. Some activity was found in the corresponding arginine carboxyl, but as will be pointed out later this doubtless arises also by CO_2-fixation.

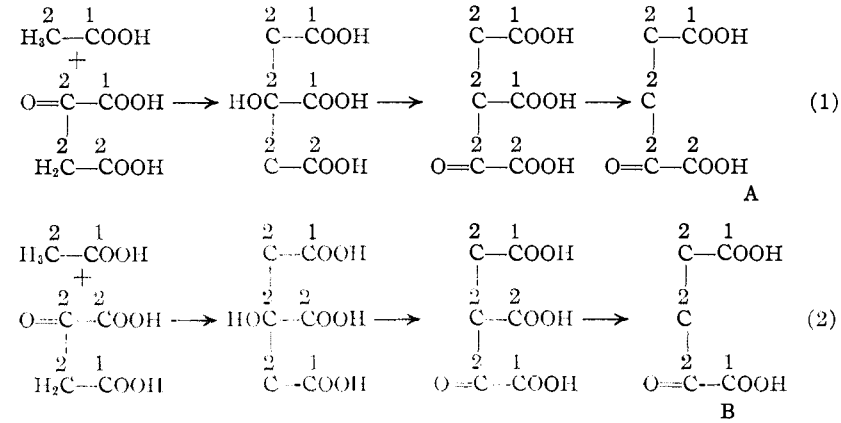
Discussion

If, as previous studies suggest, α-ketoglutarate is the source of the carbon skeleton of the ornithine moiety of arginine, both substances should have the same relative distribution of isotopic carbon. Since α-ketoglutarate is a component of the citric acid cycle the distribution therein of labeled carbon from a substance whose pathway of entry into the cycle is known can be calculated. For example, the theoretical distribution of acetate carbons in ketoglutarate can be ascertained in the following way[15]: By tracing acetate carbons through successive rounds of the cycle a steady state is reached when the 4-carbon dicarboxylic acids have three of their carbons (one carboxyl and the two α-carbons) representative of the acetate methyl carbon and have the remaining one carboxyl carbon representative of the acetate carboxyl carbon. It is assumed in this analysis (a) that citric acid undergoes asymmetric dehydration to *cis*-aconitate in such fashion that the carbonyl carbon of oxalosuccinic acid represents the methylene carbon of the oxalacetate moiety; and (b) that the regenerated 4-carbon acids have their carboxyls randomized by passage through a symmetrical intermediate (presumably succinate and fumarate).

As shown in equations 1 and 2, this type of labeled oxalacetate can condense with acetate in two ways depending on whether the carbonyl carbon is adjacent to or one carbon atom removed from the carboxyl representing the acetate carboxyl. In these equations the α- and carboxyl-carbons of acetate are designated 2 and 1, respectively.

This would lead, as shown, to two varieties of citrate, and ultimately to the two varieties of α-ketoglutarate, A and B. Since the formation of each variety of ketoglutarate is equally probable the observed distribution should be the average of these two forms and should therefore have the following characteristics: (a) twice as much acetate carboxyl carbon in the γ-carboxyl as in the α-carboxyl; (b) no acetate carboxyl carbon in the three non-carboxyl carbons; (c) equal amounts of acetate methyl carbon in all three non-carboxyl carbons; (d) one-half as much methyl carbon activity in the α-carboxyl as in the non-carboxyl carbons; and (e) no methyl carbon activity in the γ-carboxyl. These relationships (which should apply to the ornithine as well as to the α-ketoglutarate carbons) have been expressed in somewhat different form in Table III, namely, as the activity each carbon of the chain would have if the over-all activity of the five carbons of the ornithine moiety was 100. On this basis carbons 1 and 5 of the ornithine moiety produced from carboxyl-labeled acetate should have activities of 167 and 333, respectively, with none in the 2, 3 and 4 carbons. Experimentally, no activity was found in the 3 and 4 carbons. If we assume also that there is no activity in carbon-2, all of the activity in the 2 and 5 carbons can be assumed to be in the 5-position. The values thus obtained, also based on an activity of 100 for ornithine, are shown in column 4 alongside the calculated values in Table III. The two sets of values are in remarkably good agreement.

In similar fashion we can calculate that the ornithine derived from methyl-labeled acetate should have equal activities of 143 in the non-carboxyl carbons, 72 in the α-carboxyl and none in the γ-carboxyl.



(12) E. A. Evans and L. Slotin, *J. Biol. Chem.*, **136**, 805 (1940).
(13) D. Rittenberg and H. Waelsch, *ibid.*, **136**, 799 (1940).
(14) A. M. Delluva and D. W. Wilson, *ibid.*, **166**, 739 (1946).
(15) A detailed discussion of the distribution of acetate carbons in citric acid cycle components has been given in a previous paper (THIS JOURNAL, **73**, 2500 (1951)).

TABLE III

COMPARISON OF OBSERVED AND CALCULATED DISTRIBUTIONS OF LABELED CARBON AMONG ORNITHINE CARBONS[a]

Carbon atom number	Precursor: Acetate — Methyl Obsd.	Methyl Calcd.	Acetate — COOH Obsd.	COOH Calcd.	Glycine — Methylene Obsd.	Methylene Calcd.	Glycine — COOH Obsd.	COOH Calcd.	Lactate — COOH Obsd.	COOH Calcd.	Formate Obsd.	Formate Calcd.
1	68	72	170	167	131	120	500	500	359	500	500	500
2	133	143	0	0	76	72		0		0		0
3	147	143	−10	0	76	72	0[b]	0	35[b]	0	0[b]	0
4	147	143	−10	0	76	72		0		0		0
5	0	0	304	333	178	167		0		0		0

[a] These values are all calculated from the data in Table II. [b] These values are estimated from the over-all activities of the succinate moiety (carbons 2–5), which was not further degraded in these experiments.

If we assume that in this ornithine all of the activity of the 2- and 5-positions is in the 2-position we obtain the experimental values displayed in column 2 of Table III. Again the agreement between the observed and calculated distributions is gratifyingly close.

Distribution in Ornithine of Carbon from Methylene-labeled Glycine.—In a previous paper[7] it was shown that the glycine methylene carbon appeared approximately equally in all carbons of the fatty acids of the cell lipides. From previous studies which established the conversion of glycine to serine,[16,17] it was clear that the α-carbon of glycine was being incorporated in the α- and β-carbons of serine, and thence, by way of pyruvate, into both carbons of acetate, the immediate source of the labeled fatty acids. Glycine α-carbon should therefore be distributed in α-ketoglutarate in the same manner as doubly labeled acetate, and this would be the average of the distributions of methyl- and carboxyl-labeled acetate. Again a close correspondence was noted between the observed and calculated distributions of glycine α-carbon among the ornithine carbons. (In this instance the activity of carbon-5 was estimated from the 2 and 5 carbons by assuming carbon-2 has the same activity as carbons-3 and -4.) These data thus not only provide substantiation for the concept that α-ketoglutarate is the source of the ornithine carbon skeleton; they also indicate, from the high level of activity of arginine arising from the glycine α-carbon (see Table I), that metabolism *via* serine, pyruvate and the citric acid cycle represents a major glycine pathway in this strain of yeast.

Distribution of Lactate and Glycine Carboxyl Carbons and of Formate Carbon in Ornithine.—There is no direct pathway for entry of lactate carboxyl carbon in α-ketoglutarate. However, CO_2-fixation by carboxylation of carboxyl-labeled pyruvate derived from lactate oxidation should yield oxalacetate labeled in both carboxyls and should ultimately yield α-ketoglutarate labeled only in the α-carboxyl (carbon 1 of the ornithine moiety). In agreement with this formulation the ornithine moiety of arginine synthesized in the presence of carboxyl-labeled lactate was found to have essentially all of its activity in carbon 1. The small amount of activity observed in the succinate moiety (carbons 2–5) is of doubtful significance since the level is of the order of the experimental error.

Since carboxyl-labeled glycine would be expected to yield carboxyl-labeled pyruvate *via* serine, the same distribution in ornithine would be expected from the glycine carboxyl as from the lactate carboxyl. As shown in Table III, this expectation was borne out experimentally, essentially all of the activity having been found in the ornithine carboxyl.

The similar distribution of formate carbon in ornithine indicates that this conversion is not direct, but probably takes place *via* oxidation to CO_2 followed by β-carboxylation of pyruvate. In view of the known role of formate as a precursor of the β-carbon of serine,[16,17] activity was expected in the 2, 3 and 4 positions, through the intermediate formation of α-labeled acetate. Inasmuch as no activity was found in these positions, the incorporation of formate as such into serine may be presumed to have been relatively slow in these experiments.

CO_2-Fixation in Arginine.—Through the kindness of Dr. Minor Coon of the Department of Physiological Chemistry of the University of Pennsylvania, a sample of arginine was obtained, which was synthesized by yeast grown essentially as described in these experiments, but in the presence of labeled bicarbonate. This material was found to be labeled predominantly in the guanido carbon and to a lesser extent in the carboxyl. On the basis of an activity of 100 for arginine, the guanido activity was 490 and the carboxyl activity was 80. This distribution is similar to that obtained with formate and the carboxyls of lactate and glycine and hence may be considered substantiating evidence that the incorporation of these carbons into arginine results from CO_2-assimilation.

Role of Glutamate.—In experiments somewhat similar to those described in the present investigation, Ehrensvärd, *et al.*,[18,19] found that glutamic acid synthesized by an acetate-adapted strain of *Torulopsis utilis* had a distribution pattern, in its carboxyl and non-carboxyl carbons, of acetate methyl and carboxyl carbons strikingly similar to that reported here for the ornithine moiety of arginine. Though these authors did not degrade arginine extensively they pointed out[18] that the acetate methyl and carboxyl activities in the arginine carboxyl carbon, determined by means of ninhydrin, were in accord with its direct formation from glutamic acid. These data, in conjunction with the results of studies with mutant strains of

(16) P. Sickevitz and D. M. Greenberg, *J. Biol. Chem.*, **180**, 845 (1949).

(17) W. Sakami, *ibid.*, **176**, 995 (1948).

(18) G. Ehrensvärd, L. Reio and E. Saluste, *Acta Chem. Scand.*, **3**, 645 (1949).

(19) G. Ehrensvärd, L. Reio, E. Saluste and R. Stjernholm, *J. Biol. Chem.*, **189**, 91 (1951).

microörganisms[5,6] and those of the present investigation, leave hardly any doubt that the formation of arginine in *Torulopsis utilis* involves a direct conversion of α-ketoglutarate *via* glutamate, proline, ornithine and citrulline.

PHILADELPHIA 11, PENNA. RECEIVED OCTOBER 6, 1951

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF MICHIGAN]

Antispasmodics. IX. β-Diethylaminoethyl Esters of Substituted α-Aryl-β-hydroxypropionic Acids

BY F. F. BLICKE AND HAROLD RAFFELSON[1,2]

A series of substituted α-aryl-β-hydroxypropionic acids has been prepared by interaction of an Ivanov reagent, such as aryl-CH_2COONa or aryl-CH(R)COONa, with a carbonyl compound. The acids were converted into their β-diethylaminoethyl esters. A few of the esters exhibited high antispasmodic activity.

During the last few years we have prepared a variety of substituted α-aryl-β-hydroxypropionic acids by the interaction of an Ivanov reagent[3,4] with a compound which contains a carbonyl group[5]

$$\text{Aryl-CH}_2\text{COONa} \xrightarrow[(CH_3)_2CHMgCl]{}$$

R and R′ = H or hydrocarbon radical

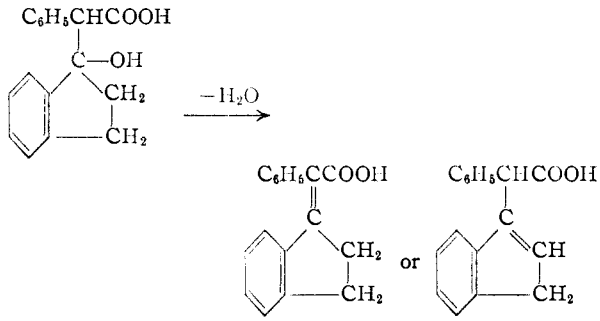
$$\text{Aryl-CH(MgCl)COONa} \xrightarrow[(2)\ HCl]{(1)\ RR'CO} \text{Aryl-CHCOOH}\ (\text{RR'COH})$$

During this investigation Ivanov reagents were prepared from the sodium salts of phenyl-, 2-thienyl-, 3-thienyl- and *p*-xenylacetic acid and from the sodium salts of α-substituted phenylacetic acids such as diphenyl-, benzylphenyl-, cyclohexylphenyl- and phenoxyphenylacetic and α-phenylbutyric acid. These reagents were allowed to react with a variety of acyclic aldehydes and ketones and with cyclic ketones such as cyclopentanone, cyclohexanone, 3-thiophanone and 1- and 2-hydrindone. The acids which were produced are reported in Tables I and II.

Isobutyraldehyde and the chloromagnesium derivative of sodium phenylacetate reacted, according to Ivanov and Nicolov,[6] to produce two compounds which seemed to be isomeric α-phenyl-β-hydroxyisocaproic acids; one acid melted at 139–140°, the other at 171–172°. When we carried out this experiment, the only product isolated was an acid which melted at 126–127°, and the neutralization equivalent found corresponded to that calculated for α-phenyl-β-hydroxyisocaproic acid.

From the interaction of benzophenone with the chloromagnesium derivative of sodium phenylacetate, an acid (m.p. 206–207°) was obtained which, based on a neutralization equivalent, is α,β,β-triphenyl-β-hydroxypropionic acid. Paterno and Chieffi[7] stated that they isolated this acid (m.p. 205–208°) after exposure of a mixture of benzophenone and phenylacetic acid to sunlight. However, when Ivanov and Spassov,[8] and later Ivanov and Ivanov,[9] employed the same reaction used by us, they claimed that they obtained α,β,β-triphenyl-β-hydroxypropionic acid which melted at 186–187°. We are unable to explain this discrepancy.

The chloromagnesium derivative mentioned above reacted with 2-hydrindone in the expected manner to produce phenyl-(2-hydroxy-2-hydrindyl)-acetic acid. When 1-hydrindone was used, an unsaturated acid was obtained. Apparently, the hydroxy acid, formed initially, underwent dehydration during the process of isolation.



When $C_6H_5CH(MgCl)COONa$ was allowed to react with ethylene oxide, and the reaction mixture acidified at 0°, the product isolated was α-phenyl-γ-hydroxybutyric acid. However, when the reaction mixture was acidified at room temperature, an oily, alkali-insoluble product, undoubtedly α-phenyl-γ-butyrolactone, was obtained.

Formaldehyde and the chloromagnesium derivative of sodium diphenylacetate reacted to form α,α-diphenyl-β-hydroxypropionic acid. Treatment of the acid with lithium aluminum hydride converted it into 2,2-diphenyl-1,3-propanediol which was identical with the diol obtained by reduction of diethyl diphenylmalonate with lithium aluminum hydride.

In some instances, as in the preparation of tropic acid,[10] it may be more convenient to employ the chloromagnesium instead of the sodium salt. The former salt was used recently by Weston and

(1) This paper represents part of a dissertation submitted by Harold Raffelson in partial fulfillment of the requirements for the Ph.D. degree in the University of Michigan.

(2) Sterling-Winthrop Fellow.

(3) D. Ivanov and A. Spassov, *Bull. soc. chim.*, [4] **49**, 19 (1931).

(4) This term may be used to designate a compound of the type aryl-CH(MgCl)COONa or aryl-CH(MgCl)COOMgCl.

(5) A paper which described the preparation of a number of acids by the use of an Ivanov reagent, as well as the preparation of basic esters of the acids, was read before the Medicinal Chemistry Division of the American Chemical Society at the Cleveland meeting, April, 1951.

(6) D. Ivanov and N. I. Nicolov, *Bull. soc. chim.*, [4] **51**, 1325 (1932).

(7) E. Paterno and G. Chieffi, *Gazz. chim. ital.*, **40**, II, 321 (1910).

(8) D. Ivanov and A. Spassov, *Bull. soc. chim.*, [4] **49**, 377 (1931).

(9) D. Ivanov and Ch. Ivanov, *Compt. rend.*, **226**, 1199 (1948).

(10) F. F. Blicke, Harold Raffelson and Bohdan Barna, THIS JOURNAL, **74**, in press (1952).