THE DEGRADATION OF α-AMINOADIPIC ACID IN GUINEA PIG LIVER HOMOGENATE*

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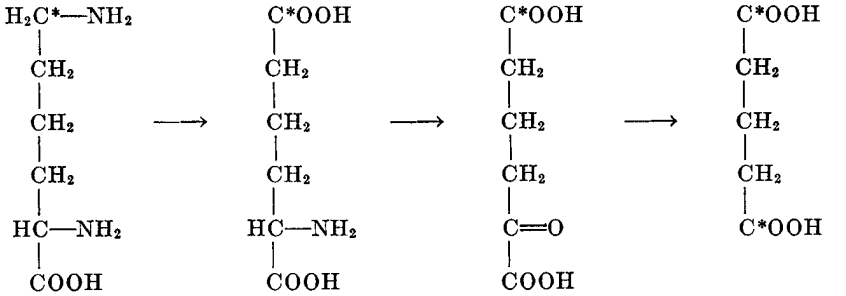
In continuation of our study of the metabolism of L-lysine, α-aminoadipic acid, which is formed from lysine in guinea pig liver homogenate (1), was synthesized with C^{14} in the ε-position. The metabolism of the latter compound was followed by search for the radioactive tracer among the probable metabolic products. Two have been identified, α-ketoadipic and glutaric acids.

The accompanying diagram (I) indicates one of the pathways of the catabolism of lysine. The asterisk indicates the position of the labeled carbon.



(I)

Preparations

A summary of the synthesis and resolution of α-aminoadipic acid labeled with C^{14} in the ε position is shown in diagram (II). The radioactive carbons are marked with an asterisk.

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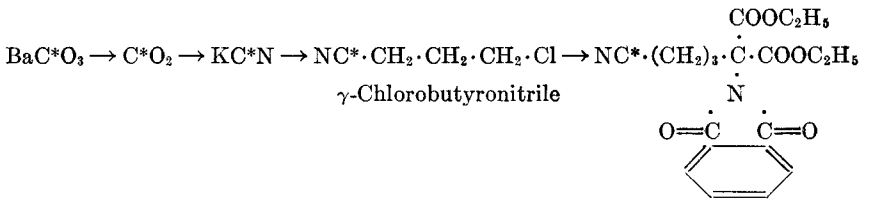
The C^{14} used in this investigation was supplied by the Monsanto Chemical Company, Clinton Laboratories, Oak Ridge, Tennessee, and obtained on allocation from the United States Atomic Energy Commission.

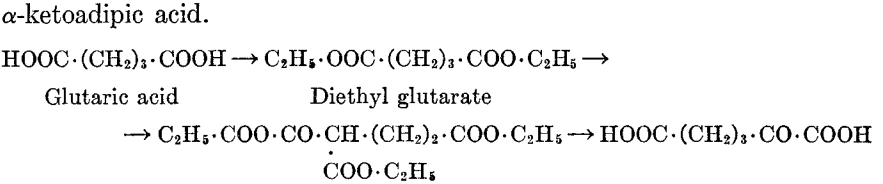


(II)

The details of the preparation have been described in a previous communication (1).

89.3 mg. of $BaC^{14}O_3$ containing 0.625 millicurie of C^{14} yielded 0.127 gm. of pure L-α-aminoadipic acid, giving 14,450 counts (corrected) per mg. per minute.

α-Ketoadipic Acid—Diagram (III) is a summary of the synthesis of α-ketoadipic acid.



(III)

Diethyl glutarate was prepared by the method of Locquin and Elghozy (2), and triethyl 2-oxaloglutarate by the method of Gault (3). The latter compound was decarboxylated with HCl. After removal of the HCl *in vacuo* spontaneous crystallization set in. The crude product was purified by solution in warm ether and reprecipitation by the addition of an equal volume of petroleum ether. The light orange crystals had the following composition.

$C_6H_8O_5$ (160.06). M.p. 124°. Calculated, C 44.98, H 5.04; Found, C 45.12, H 5.04

Procedure

The preparation of the guinea pig liver homogenate and the saline solution are described in a previous communication (1).

In a representative experiment 2 ml. of homogenate containing 0.66 gm. of liver (wet weight) and 2 ml. of saline solution containing a mixture of 5 mg. of L-α-aminoadipic acid (14,450) counts (corrected) per minute per mg.) and 5 mg. of non-radioactive L-α-aminoadipic acid were added to

each of four 20 ml. beakers. Two were immediately brought to pH 5.0 and boiled for 10 minutes, the coagulated protein extracted with water, and the non-protein filtrates and washings combined. The other two beakers were incubated at pH 7.4 under oxygen at 38° for 6 hours, then deproteinized by boiling at pH 5.0, and their non-protein filtrates combined.

Results

The non-protein filtrates were cleared by boiling with charcoal, acidified with hydrochloric acid to a concentration of 0.1 N, and then extracted with ether. After the ether was evaporated, the residue was taken up in 3 ml. of water; 100 mg. of non-radioactive α-ketoadipic acid and 91 mg. of phenylhydrazine hydrochloride dissolved in 2 ml. of water were added. The oil, α-ketoadipic acid phenylhydrazone, which settled out first, crystallized in 2 hours. The recrystallization procedure was as follows: 2 ml.

TABLE I

Specific Radioactivity of α-Ketoadipic Acid Phenylhydrazone after Successive Recrystallizations

No. of crystallizations	Counts (corrected) per mg. per min. of phenylhydrazone from	
	Reaction mixture at zero time	Reaction mixture after 6 hrs. incubation
1	1.8	10.1
2	1.0	3.1
3	0	2.7
4	0	2.6
5	0	2.7
6	0	2.8

of water were added to the crystals, and the suspension brought to boil and then treated with ethanol dropwise until all the crystals dissolved. The solution was then cooled, the crystals collected, and their specific radioactivity determined.

Table I gives the specific radioactivities of the phenylhydrazone samples obtained from the reaction mixtures at zero time and after 6 hours incubation. The figures show that by the second recrystallization all the radioactivity had been removed from the phenylhydrazone obtained from the zero time reaction mixture, and that constant specific activity had been attained in the case of the phenylhydrazone obtained after 6 hours incubation. The counts in the first and second crystallizations probably arose from α-aminoadipic acid, and, in the case of the 6 hour reaction mixture, also from glutaric acid arising from degradation of the α-ketoadipic acid formed from the added α-aminoadipic acid.

The melting point of the radioactive phenylhydrazone after the fifth crystallization was 141–142°, and of a mixture with an authentic sample 141–142°. Gault (3) reported the melting point as 141°. After the final recrystallization, 15 mg. of the phenylhydrazone remained. 90 per cent of the added α-ketoadipic acid was, therefore, left in the mother liquors.

These data prove that the α-aminoadipic acid was oxidatively deaminized to α-ketoadipic acid.

The rate of formation of α-ketoadipic acid from α-aminoadipic acid can be calculated from the value of the constant specific activity of the α-ketoadipic acid phenylhydrazone obtained from the reaction mixture incubated for 6 hours. The specific activity of the phenylhydrazone was 2.7 counts (corrected) per mg. per minute. 100 mg. of α-ketoadipic acid, equivalent to 156.3 mg. of its phenylhydrazone, were added as a carrier. The weight of the radioactive form being neglected, the total α-ketoadipic

TABLE II

Specific Radioacitivity of Barium Glutarate (Dried at 100°) after Successive Reprecipitations

No. of precipitations	Counts (corrected) per mg. per min. of barium glutarate from	
	Reaction mixture at zero time	Reaction mixture after 6 hrs. incubation
1	0	1.50
2	0	1.33
3		1.30
4		0.95
5		1.01
6		1.01

acid in the solution contained 156.3 × 2.7 = 422 counts (corrected). 144,500 counts (corrected) were added originally as 20 mg. of α-aminoadipic acid. 0.29 per cent of the latter was found as the keto acid, or 0.058 mg. Expressed as a Q value,[1] this rate is 0.005. It is about one-twelfth that of the formation of α-aminoadipic acid from L-lysine (1).

In another experiment similar to the preceding, the non-protein filtrate was examined for evidence of formation of glutaric acid. The procedure was the same as before to the stage after evaporation of the ethereal extract of the non-protein filtrate. 100 mg. of non-radioactive glutaric acid were added as a carrier in the subsequent crystallization. The mixture was taken up in 3 ml. of water and treated with saturated barium hydroxide

[1] Q is the rate of change of the substance in question expressed as if it were a gas in c.mm., at standard temperature and pressure, per mg. of dry weight of tissue used per hour.

solution to pH 9.0; the final volume was 5 ml. No precipitation occurred. 10 ml. of 95 per cent ethanol were then added. The precipitate of barium glutarate pentahydrate was filtered off. 96 per cent of the water of hydration was driven off by drying at 100°, and the specific radioactivity of the compound was determined.

The solution in water and precipitation with ethanol were repeated five times. Table II gives the specific radioactivities of the barium glutarate samples obtained as above from the reaction mixtures at zero time and after 6 hours incubation. After the final reprecipitation 30 mg. of barium salt (as pentahydrate) remained, corresponding to 11 per cent of the original glutaric acid added.

Analysis of the radioactive barium salt after the last precipitation gave, on an anhydrous basis, 51.3 per cent of barium (calculated, 51.4 per cent).

These data prove that glutaric acid was one of the products formed in the liver homogenate from the added α-aminoadipic acid. There can be little doubt that α-ketoadipic acid was formed first, and that the glutaric acid arose by its oxidative decarboxylation.

The calculation of the rate of formation of glutaric acid from the added α-aminoadipic acid is as follows: the final constant specific activity of the barium glutarate was 1.0 count (corrected) per mg. per minute; 100 mg. of glutaric acid, equivalent to 202.5 mg. of anhydrous barium glutarate, were added as carrier; the glutaric acid formed from the added α-aminoadipic acid contained, therefore, 202 counts (corrected) per minute; 144,500 counts were contained in the α-aminoadipic acid; 0.14 per cent of the latter was, therefore, found as glutaric acid. The Q value is 0.0024.

Of the two successive oxidative steps in the degradation of α-aminoadipic acid, deamination followed by decarboxylation, the latter is probably the faster. 0.43 per cent of the added α-aminoadipic acid was found in the above two degradation products. This figure represents the rate of its deamination. The corresponding rate of decarboxylation was 0.14/0.43 or 33 per cent.

SUMMARY

1. The synthesis of α-ketoadipic acid is described.

2. In guinea pig liver homogenate α-aminoadipic acid is oxidatively deaminized to α-ketoadipic acid and the latter is oxidatively decarboxylated to glutaric acid.

3. The deamination of α-aminoadipic acid is much slower than its formation from L-lysine. The decarboxylation of α-ketoadipic acid is faster than the deamination of α-aminoadipic acid.

4. The foregoing evidence was obtained with the use of lysine and of α-aminoadipic acid labeled with C^{14} in the ε position.

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