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Synthesis of 5-Hydroxy-L-tryptophan Utilizing N-Acetyl-L-glutamic γ-Semialdehyde, an Intermediate in the Metabolism of L-Glutamic Acid to L-Ornithine

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N-Acetyl-L-glutamic γ-semialdehyde was enzymatically prepared in 51% yield from N^{α}-acetyl-L-ornithine. By the reaction with 4-benzyloxy(or methoxy)-phenylhydrazine this aldehyde was converted into N-acetyl-5-benzyloxy(or methoxy)-L-tryptophan which is known to be transformed into 5-hydroxy-L-tryptophan, a biogenetic precursor of serotonin. By this work an amino acid of L-glutamic acid family was first chemically derived to that of L-tryptophan series.

5-Hydroxy-L-tryptophan (**8**) is an extremely important intermediate in the biogenetic synthesis of the intracerebral amine type hormones such as 5-hydroxytryptamine(serotonin), 5-methoxytryptamine and N-acetyl-5-methoxytryptamine(melatonin) from L-tryptophan in a living body. Further, it is useful as an antidepressant and has hormonal actions against Down's syndrome(Mongolism) and the like diseases.[1,2] The compound **8** was prepared by the optical resolution of N-acyl-5-benzyloxy-DL-tryptophan[3,4] or by the oxidation of 2,3-dihydro-L-tryptophan.[5] In the present paper we report a new synthesis of 5-hydroxy-L-tryptophan (**8**) from enzymatically prepared N-acetyl-L-glutamic γ-semialdehyde (**3**).[6]

In some bacteria[7~9] and yeast[10] N-acetyl-L-glutamic γ-semialdehyde (**3**) is present as an intermediate in the metabolism of L-glutamic acid to L-ornithine while L-glutamic γ-semialdehyde is a biogenetic precursor of L-proline. We were interested in the N-protected L-glutamic γ-semialdehyde and intended to synthesize 5-hydroxy-L-tryptophan (**8**) from it because a variety of synthetic N-protected glutamic γ-semialdehyde derivatives are known to be converted into optically inactive 5-hydroxytryptophan derivatives by the Fischer indole synthesis.[1,11]

The aldehyde **3** was prepared in 51% yield from N^{α}-acetyl-L-ornithine (**1**) and α-ketoglutaric acid (**2**) with the cell homogenate of *Corynebacterium glutamicum* (syn. *Micrococcus glutamicus*)[8] containing acetylornithine aminotransferase (EC 2.6.1.11).[12] Fisher cyclization reaction of **3** and 4-benzyloxyphenylhydrazine (**4**) in aqueous acetic acid gave N-acetyl-5-benzyloxy-L-tryptophan (**6**) in 88% yield after chromatography. Recrystallization afforded **6** of mp 198～199.5°C and $[\alpha]_D$ +6.3° (reported values[4] are mp 196.5～198°C and $[\alpha]_D$ +6.3°). The product **6** is known to be converted into **8** by the catalytic hydrogenation followed by the treatment with acid.[4]

A similar reaction of **3** and 4-methoxyphenylhydrazine (**5**) furnished N-acetyl-5-methoxy-L-tryptophan (**7**) in 65% yield. Recrystallization gave **7** of mp 173～175°C and $[\alpha]_D$ +14.3°. The authentic sample **7** which was prepared by the acetylation of fermentatively produced 5-methoxy-L-tryptophan[2] showed mp 173～174°C and $[\alpha]_D$ +14.7°.

Meanwhile, a similar reaction of **3** with phenylhydrazine (**9**) produced N-acetyl-L-tryptophan (**10**) in 31% yield.

To the best of our knowledge, an amino acid of the L-glutamic acid family was first chemically derived to that of the L-tryptophan series by this work. Moreover, this work suggests a possible synthesis of useful compounds by the

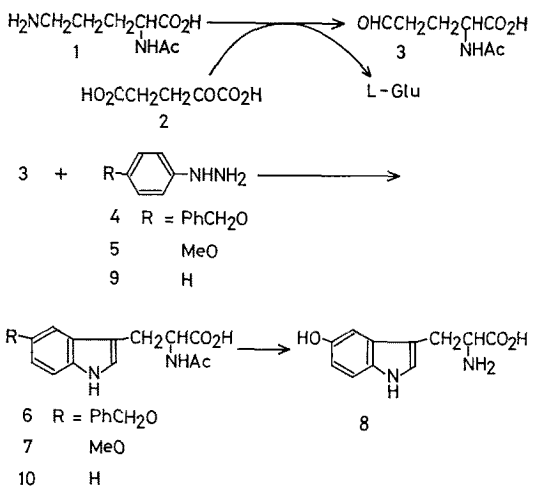

FIG. 1. Enzymatic Synthesis of N^{α}-Acetyl-L-glutamic γ-Semialdehyde (**3**) and Its Conversion into 5-Hydroxy-L-tryptophan (**8**).

combination of chemical and biochemical methods, utilizing intermediates in the biosynthetic pathways. It is well known that L-glutamic acid and L-ornithine are fermentatively produced. Therefore, it is interesting if the intermediate **3** between them is produced similarly. In fact **3** was isolated in 10 mg per liter from a culture filtrate of *Escherichia coli* strain 160-37.[7]

EXPERIMENTAL

Melting points were taken on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured on a Hitachi EPI-G3 spectrometer. The cell homogenate of *C. glutamicum* was prepared by Dr. Kazumi Araki using its arginine-requiring mutant according to the published method.[8] N^{α}-Acetyl-L-ornithine was prepared in a conventional way from N^{δ}-benzyloxycarbonyl-L-ornithine (purchased from Kokusan Chemical Works) by the acetylation with acetic anhydride in the presence of aqueous sodium hydroxide followed by the catalytic hydrogenation.

Enzymatic preparation of N-acetyl-L-glutamic γ-semialdehyde (**3**)

A solution of α-ketoglutric acid (1.17 g, 8 mmol) and N^{α}-acetyl-L-ornithine (1.39 g, 8 mmol) in water (80 ml) was adjusted to pH 7.8 with 1 N KOH (*ca.* 16.5 ml). To this solution were added 0.2 M K_2HPO_4 (40 ml) of pH 7.8, pyridoxal-5'-phosphate·H_2O (2 mg) and the cell homogenate (15 ml) of *C. glutamicum*. The mixture was stirred at 36°C for 15 hr. It was cooled with ice water, brought to pH 3 with 6 N H_2SO_4 and extracted with butanol eight times. The extract was dried (Na_2SO_4) and concentrated to an oily residue. The residue was dissolved in water (5 ml) and slowly passed through 5 g of Amberlite IR-45 (HCl form). The resin was washed with water (100 ml). The combined filtrates were concentrated at 50°C with a water aspirator to give an oil (1.095 g). This oil which is free from α-ketoglutaric acid by NMR (D_2O) still contains water and becomes glassy when dried under vacuum overnight.

A portion (84 mg) of this oil was treated with 2,4-dinitrophenylhydrazine-sulfuric acid to produce 111 mg (54 mg as **3**) of the correrponding hydrazone. From this result, the yield of **3** was estimated to be 51%. Recrystallization of the hydrazone from methanol gave mp 205~207°C (reported value[7,8] is mp 208°C).

Synthesis of N-acetyl-5-benzyloxy-L-tryptophan (**6**)

A stirred suspension of 91 mg (0.34 mmol as **3**) of the oil obtained above and 4-benzyloxyphenylhydrazine·HCl[13] (79 mg, 0.315 mmol) in a mixture of water (3 ml) and acetic acid (4 ml) was heated gradually from room temperature to 80°C over 1 hr. At 80°C the reaction was stopped and the mixture was concentrated giving a residue. Water was added to the residue, and the mixture was extracted with ethyl acetate. The extract was dried, concentrated and dissolved in ethyl acetate–dichloromethane (4: 6). The solution was applied to a silica gel (Wakogel C-200) column which was prepared with dichloromethane. Elution with ethyl acetate–dichloromethane (4: 6) afforded **6** (98 mg, 88%) of $[\alpha]_D^{26}$ +6.0° (c=0.98, MeOH) which crystallized on standing. Recrystallization from ethyl acetate-cyclohexane gave 85 mg; mp 198~199.5°C and $[\alpha]_D^{26}$ +6.3° (c=0.80, MeOH).

Synthesis of N-acetyl-5-methoxy-L-tryptophan (**7**)

A stirred suspension of 148 mg (0.55 mmol as **3**) of the above oil and 90 mg (0.51 mmol) of 4-methoxyphenylhydrazine·HCl (purchased from Aldrich, recrystallized from methanol) in a mixture of water (3 ml) and acetic acid (2 ml) was heated from room temperature to 80°C over 40 min and then heated at 80°C for 20 min. The mixture was worked up and chromatographed in the same manner as the case of **6** to produce 121 mg (65%) of **7**, $[\alpha]_D^{23}$ +11.8° (c=1, MeOH), which crystallized on standing. Two times recrystallization from ethyl acetate-cyclohexane gave **7** of mp 173~175°C and $[\alpha]_D^{23}$ +14.3° (c=0.725, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1215, 1240, 1535, 1625, 1730, 2200~3100, 3200~3450. Found: C, 60.87; H, 5.93. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.84; H, 5.84%.

Preparation of **7** *from fermentatively produced 5-methoxy-L-tryptophan*

To a stirred solution of 5-methoxy-L-tryptophan[2]

(95 mg, 0.406 mmol) in 0.1 N NaOH (2.5 ml) was added acetic anhydride (0.02 ml, 0.21 mmol), and the mixture was stirred for 20 min. To the mixture were added 0.5 N NaOH (0.5 ml) and acetic anhydride (0.06 ml, 0.63 mmol), and the mixture was stirred for 30 min. The mixture was brought to pH 8~9 with 1 N $NaHCO_3$ (2 ml), and it was washed with ethyl acetate. The aqueous layer was acidified with 2 N HCl and extracted with ethyl acetate. The extract was dried, concentrated and crystallized from ethyl acetate-cyclohexane. Recrystallization from the same solvent system furnished 83 mg (74%) of **7**; mp 173~174°C and $[\alpha]_D^{23}$ +14.7° (c=0.79, MeOH).

Synthesis of N-acetyl-L-tryptophan (**10**)

A stirred suspension of 81 mg (0.33 mmol as **3**) of the oil obtained above and phenylhydrazine·HCl (68 mg, 0.47 mmol) in acetic acid (4 ml) was heated from room temperature to 80°C over 40 min and then heated at 80°C for 1 hr. The mixture was worked up and chromatographed as in the case of **6** to produce 25 mg (31%) of **10** which partially crystallized on standing. The compound showed $[\alpha]_D^{26}$+21.6° (c=0.5, MeOH) while the authentic sample (purchased from Tokyo Kasei Kogyo Co., Ltd.) had $[\alpha]_D^{26}$+22.6° (c=1, MeOH).

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