[Chem. Pharm. Bull. 32(9)3620—3625(1984)]

# Comparative Effects of DL, D and L-3-Pyridylalanine on Serotonin Concentration and Tryptophan–Serotonin Metabolizing Enzymes

HIROSHI SHIMENO,* YOKO FUKUMOTO, MASAKATSU FUJI and ATSUO NAGAMATSU

*Faculty of Pharmaceutical Sciences, Fukuoka University, 19–1 Nanakuma 8-chome, Jonan-ku, Fukuoka 814–01, Japan*

(Received January 11, 1984)

The administration of DL-3-pyridylalanine (DL-3-PA, DL-α-amino-3-pyridinepropanoic acid) or L-3-PA significantly increased brain serotonin (5-HT) concentration without affecting the other tissue 5-HT concentrations in male Wistar rats. The increase in brain 5-HT upon the administration of DL or L-3-PA was maintained for a long time (at least 96 h). DL and L-3-PA decreased tryptophan pyrrolase activity in the liver and increased free tryptophan concentration in the serum. These effects found in rats given a 100 mg/kg dose of DL-3-PA were not statistically different from those in rats given a 50 mg/kg dose of L-3-PA. DL and L-3-PA hardly affected the activities of L-tryptophan 5-hydroxylase, 5-hydroxy-L-tryptophan decarboxylase and monoamine oxidase in the brain. D-3-PA had no effect on 5-HT concentration or tryptophan-5-HT metabolizing enzymes in rats. These results suggest that the action of DL-3-PA is due to L-3-PA, not D-3-PA.

**Keywords**——DL-3-pyridylalanine; L-3-pyridylalanine; D-3-pyridylalanine; increasing effect on brain serotonin; tryptophan pyrrolase; serum free tryptophan

We have previously reported that DL-3-pyridylalanine (DL-3-PA) decreased liver tryptophan pyrrolase (TP, EC 1.13.11.11) activity in rats, and that this compound increased serum free tryptophan and brain serotonin (5-HT) concentrations.[1] Furthermore, we found that the tryptophan-induced increase in liver TP activity was clearly prevented by DL-3-PA and that brain 5-HT concentration was effectively increased by the combined administration of L-tryptophan with DL-3-PA as compared with single administration of L-tryptophan.[2] On the basis of these results, we suggested that the TP inhibitor, DL-3-PA, might increase the therapeutic effect of L-tryptophan on depressive illness and that the increase in brain 5-HT upon the administration of DL-3-PA occurred *via* the inhibition of liver TP. It is therefore of interest to find out which isomer of 3-PA is active.

The present paper describes the comparative effects of DL, D and L-3-PA on the concentration of 5-HT and on the activities of some tryptophan-5-HT metabolizing enzymes in rats.

## Methods

**Chemicals**——DL-3-PA, mp 261—262 °C, was synthesized by the method of Nieman *et al.*[3] The commercial sources of most chemicals have been previously described.[1] Porcine kidney acylase I (EC 3.5.1.14, 5000—8000 units/mg protein) was obtained from Sigma, St. Louis, Mo., U.S.A. All other chemicals were of the purest commercially available grade from Wako Pure Chemicals, Osaka.

**Resolution of DL-3-PA**——DL-3-PA was resolved by a modification of the method of Greenstein and Winitz,[4] who reported the enzymic resolution of DL-alanine. *N*-Acetyl-DL-3-PA (0.1 mol), which was prepared by acetylation of DL-3-PA, was incubated with 20 mg of porcine kidney acylase I at pH 7.0—7.2 for 24 h (25 °C). The reaction mixture was concentrated to a small volume under reduced pressure. On addition of absolute ethanol to 80% (v/v), L-

NII-Electronic Library Service

3-PA immediately precipitated. After chilling of the mixture, the amino acid was filtered off with suction, washed successively with ethanol and ether, and then dried. The crude compound was recrystallized from 80% aqueous ethanol to give L-3-PA ($\alpha_D^{25} = -9.73$ in $H_2O$, 1% (w/v) solution). The filtrate was evaporated to dryness. The residue (*N*-acetyl-D-3-PA) was hydrolyzed with concentrated hydrochloric acid on a boiling water bath. The hydrochloric acid was removed by repeated evaporation after the addition of water, and the residue was dissolved in a small volume of water. On addition of absolute ethanol to 80%, the crude D-3-PA crystallized rapidly. The crystals were filtered off with suction, washed successively with ethanol and ether, and dried. The crude amino acid was recrystallized as described above for the L form to give D-3-PA ($\alpha_D^{25} = +9.67$ in $H_2O$, 1% solution).

**Animals and Drug Administration**——Male Wistar rats (140—160 g, Kyudo Co., Kumamoto) were subcutaneously injected with a solution of DL, D or L-3-PA in 0.9% (w/v) NaCl. Control animals were given saline alone. The animals were killed by decapitation at the same time of day (14:00–14:30) to obviate possible errors due to circadian variation. Brain and the other tissues were dissected out rapidly, washed in ice-cold 0.9% NaCl, blotted dry and immediately used for assay. For the measurement of free tryptophan in the serum, the protein-bound fraction was removed from a portion of each serum sample by ultrafiltration with an Amicon PM-10 membrane. The ultrafiltrate was immediately used for the measurement.

**Analytical Methods**——5-HT in the tissues, free tryptophan in the serum, L-tryptophan pyrrolase (TP), L-tryptophan 5-hydroxylase (EC 1.14.16.4), 5-hydroxy-L-tryptophan decarboxylase (5-HTP decarboxylase, EC 4.1.1.28) and monoamine oxidase (MAO, EC 1.4.3.4) were determined as described previously.[1]

Statistical analysis of results was performed by the use of Student's *t*-test.

## Results

### Effects of DL, D and L-3-PA on Brain 5-HT Concentration

The effects of DL, D and L-3-PA given at four doses on the concentration of 5-HT in the brain are shown in Fig. 1a. In agreement with previous results,[1] brain 5-HT concentration was significantly increased by a 100 mg/kg dose of DL-3-PA. The administration of L-3-PA resulted in a dose-related increase in brain 5-HT concentration. The degree of increase in brain 5-HT after a 50 mg/kg dose of L-3-PA was not statistically different from that after a

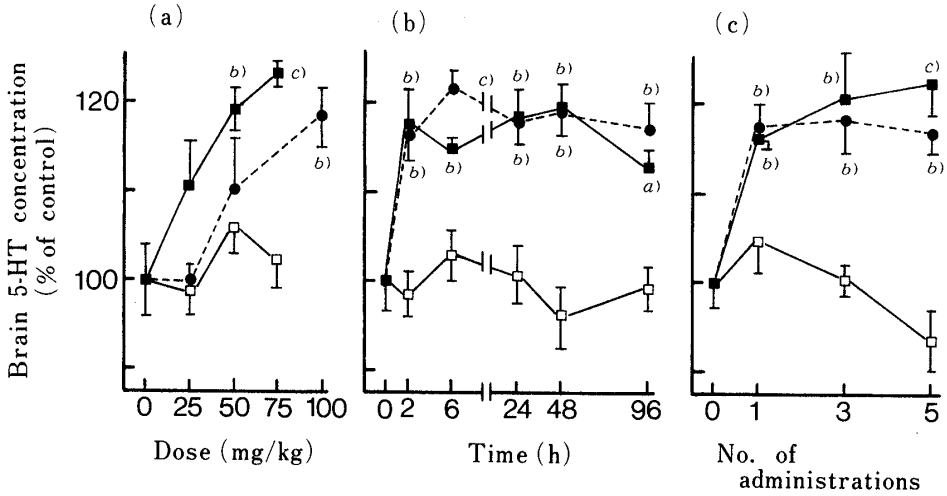


Fig. 1. Effects of DL, D and L-3-Pyridylalanine (3-PA) on Brain Serotonin (5-HT) Concentration

a) Rats were given a subcutaneous injection of saline or various doses of DL- (●---●), D- (□—□) or L-3-PA (■—■). Brain 5-HT concentration was determined at 24 h after the administration.

b) Rats were subcutaneously injected with saline or 3-PA (D and L-3-PA, 50 mg/kg; DL-3-PA, 100 mg/kg) and sacrificed at the time indicated.

c) Control rats were injected with saline. When 3-PA (D and L forms, 50 mg/kg; DL form, 100 mg/kg) was given repeatedly, subcutaneous injection was carried out once daily. Brain 5-HT concentration was determined at 24 h after the last injection.

Each point represents the mean ± S.E. for 6 animals per group.

Significance of differences from controls; *a*) $p<0.02$, *b*) $p<0.01$, *c*) $p<0.001$.

Control values are as follows; 5-HT μg/g wet wt.; (a), 0.402 ± 0.016; (b), 0.426 ± 0.015; (c), 0.383 ± 0.011.

NII-Electronic Library Service

100 mg/kg dose of DL-3-PA. On the other hand, the D-form hardly affected the concentration of 5-HT in the brain.

The time-courses of the changes in brain 5-HT concentration after single administrations of DL, D and L-3-PA are shown in Fig. 1b. L-3-PA (50 mg/kg) caused a significant increase of 5-HT in the brain at 2 h after the administration, and the effect was still apparent at 96 h. The effect of DL-3-PA (100 mg/kg) was similar to that of L-3-PA (50 mg/kg). D-3-PA (50 mg/kg) did not increase the concentration of 5-HT in the brain at any of the time-intervals examined.

As shown in Fig. 1c, repeated daily administrations of DL-3-PA (100 mg/kg) did not produce any further increase in brain 5-HT concentration as compared with that found in animals given a single dose of DL-3-PA. In case of L-3-PA (50 mg/kg/d), brain 5-HT concentration after the chronic administration was higher than that the administration of DL-3-PA (100 mg/kg/d), but the difference between the two was not statistically significant. Chronic administration of D-3-PA (50 mg/kg/d) did not cause a significant increase in brain 5-HT.

**Effects of DL, D and L-3-PA on Liver, Kidney, Spleen and Small Intestine 5-HT Concentrations**

Liver, kidney, spleen and small intestine 5-HT concentrations after a single administration of DL-3-PA (100 mg/kg), D-3-PA (50 mg/kg) or L-3-PA (50 mg/kg) were not different from those in control animals (data not shown). As shown in Table I, the chronic administration (5 d) of DL-3-PA (100 mg/kg/d) or L-3-PA (50 mg/kg/d) slightly increased the small intestine 5-HT levels. However, the increases were not significant as compared with the control group. Liver, kidney and spleen 5-HT concentrations remained unchanged after the chronic administration of DL, D or L-3-PA.

**Effects of DL, D and L-3-PA on Tryptophan-5-HT Metabolizing Enzymes**

**a) Liver TP**——As shown in Fig. 2a, liver TP activity was significantly decreased in proportion to the dose of L-3-PA. DL-3-PA also resulted in a dose-related decrease in liver TP activity. The decrease was significant at 100 mg/kg, but not at 50 mg/kg dose. D-3-PA hardly affected liver TP activity at any of the doses examined.

The time-course of the effects of single administration of DL, D or L-3-PA on liver TP activity is shown in Fig. 2b. A significant decrease in liver TP activity was observed at 2 h after the administration of DL-3-PA (100 mg/kg) or L-3-PA (50 mg/kg). The effects of DL and L-3-

Table I. Effects of Repeated Daily Administration of DL, D and L-3-Pyridylalanine (3-PA) on Serotonin (5-HT) Concentrations in Various Tissues

| Group | No. of administrations | 5-HT μg/g wet wt. | | | |
|---|---|---|---|---|---|
| | | Liver | Kidney | Spleen | Small intestine |
| Control | | 0.508±0.019 | 0.290±0.007 | 3.35±0.15 | 5.89±0.18 |
| L-3-PA (50 mg/kg) | 1 | 0.523±0.027 | 0.281±0.014 | 3.48±0.23 | 5.91±0.14 |
| | 3 | 0.501±0.018 | 0.289±0.013 | 3.40±0.20 | 6.10±0.20 |
| | 5 | 0.543±0.025 | 0.305±0.009 | 3.33±0.17 | 6.41±0.21 |
| D-3-PA (50 mg/kg) | 1 | 0.509±0.021 | 0.288±0.013 | 3.37±0.18 | 6.07±0.19 |
| | 3 | 0.519±0.023 | 0.285±0.007 | 3.49±0.26 | 6.04±0.23 |
| | 5 | 0.502±0.029 | 0.294±0.011 | 3.44±0.12 | 6.18±0.33 |
| DL-3-PA (100 mg/kg) | 1 | 0.490±0.026 | 0.306±0.008 | 3.29±0.11 | 5.95±0.16 |
| | 3 | 0.521±0.015 | 0.277±0.008 | 3.43±0.17 | 6.18±0.15 |
| | 5 | 0.519±0.042 | 0.299±0.012 | 3.21±0.22 | 6.48±0.26 |

The tissue 5-HT concentrations were determined at 24 h after the last administration. When 3-PA was given repeatedly, the administration was carried out once daily. Values represent the mean±S.E. for 6 animals per group.

NII-Electronic Library Service

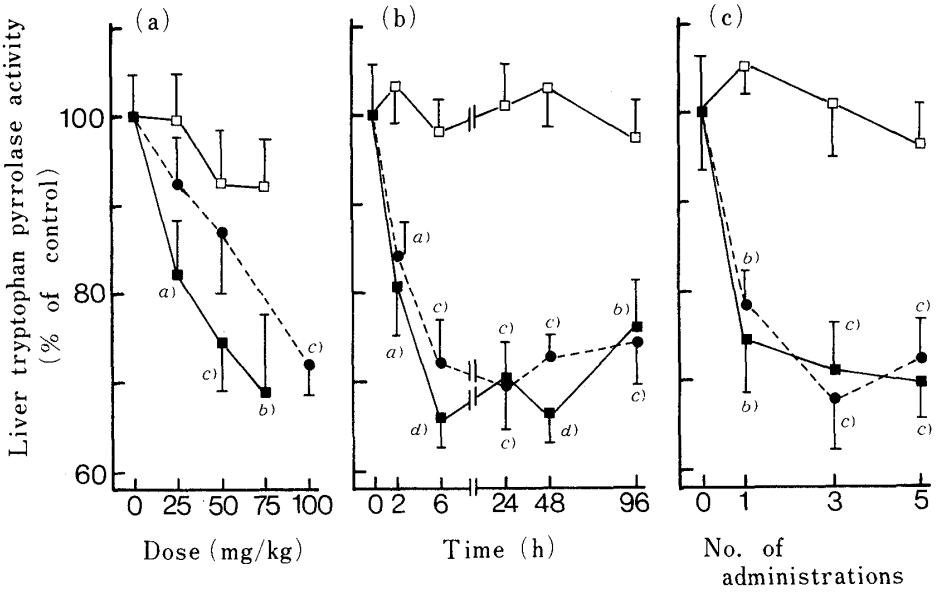


Fig. 2. Effects of DL, D and L-3-Pyridylalanine (3-PA) on Liver Tryptophan Pyrrolase (TP) Activity

Rats were injected DL- (●---●), D- (□—□) or L-3-PA (■—■) as described in the legend to Fig. 1.

Each point represents the mean ± S.E. for 6 animals per group.

Significance of differences from controls; *a)* $p<0.05$, *b)* $p<0.02$, *c)* $p<0.01$, *d)* $p<0.001$.

Control values are as follows; Kynurenine μmol/g wet wt./h: (a), 8.44±0.40; (b), 8.68±0.50; (c), 9.06±0.58.

PA were maintained throughout the duration of the experiment. There was statistically no difference in the degree of the decrease in liver TP activity between the DL and L forms. D-3-PA (50 mg/kg) did not decrease liver TP activity at any time interval examined.

The enzyme activity in chronically L-3-PA (50 mg/kg/d)-treated animals was lower than that in rats singly administered L-3-PA, but the difference between the two was not statistically significant (Fig. 2c). The chronic administration of DL-3-PA (100 mg/kg/d) did not produce a significant decrease in liver TP activity as compared with that found in animals given a single dose of DL-3-PA. Liver TP activity was not affected by chronic administration of D-3-PA (50 mg/kg/d).

**b) Brain L-Tryptophan 5-Hydroxylase**——As shown in Fig. 3-i, single and choronic administrations of D or L-3-PA did not produce a significant change in brain L-tryptophan 5-hydroxylase activity. DL-3-PA also had no effect on the enzyme activity (data not shown).

**c) Brain 5-HTP Decarboxylase**——A single administration of D or L-3-PA hardly affected brain 5-HTP decarboxylase activity (Fig. 3-iia, b). The enzyme activity was decreased slightly by chronic administration of L-3-PA (50 mg/kg/d, 5 d), but not significantly (Fig. 3-iic). On the other hand, chronic administration of DL-3-PA (100 mg/kg/d, 5 d) produced a significant decrease in the enzyme activity as compared with the control.

**d) Brain MAO**——As shown in Fig. 3-iii, brain MAO activity was not influenced by the administration of D or L-3-PA.

**Effects of DL, D and L-3-PA on the Concentration of Free Tryptophan in the Serum**

The effects of the three forms of 3-PA on the concentration of serum free tryptophan were examined at four doses and the results are shown in Fig. 4a. Single administration of L-3-PA led to an elevation of free tryptophan in the serum at all doses. The effect was significant at the 50 and 100 mg/kg doses, but not at the 25 mg/kg dose of L-3-PA. The degree of increase in serum free tryptophan after a 50 mg/kg dose of L-3-PA was not statistically different from that

NII-Electronic Library Service

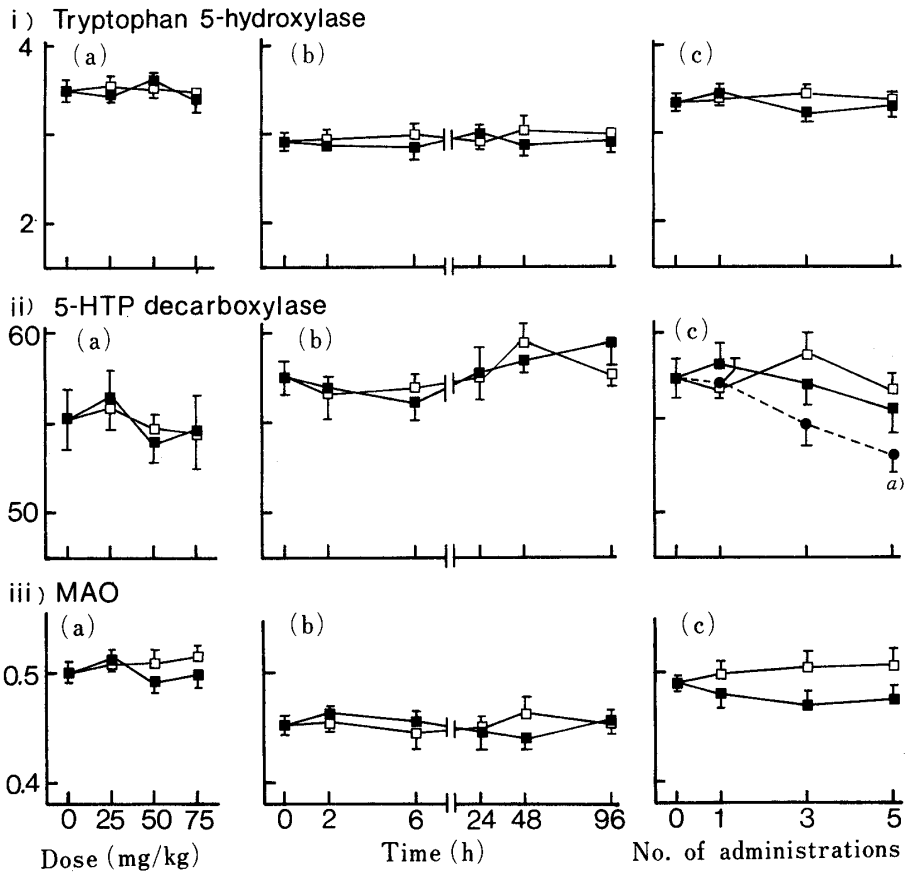


Fig. 3. Effects of DL, D and L-3-Pyridylalanine (3-PA) on the Activities of Three Enzymes Involved in Serotonin (5-HT) Metabolism in the Brain

Rats were injected with DL- (●---●), D- (□—□) and L-3-PA (■—■) as described in the legend to Fig. 1.
Each point represents the mean ± S.E. for 6 animals per group.
Enzyme activities are expressed as follows;
i) Tryptophan 5-hydroxylase: 5-HTP μg/g wet wt./15 min.
ii) 5-Hydroxytryptophan (5-HTP) decarboxylase: 5-HT μg/g wet wt./h.
iii) Monoamine oxidase (MAO): 5-HT mg/g wet wt./30 min.
Significance of differences from controls; *a*) $p < 0.05$.

after a 100 mg/kg dose of DL-3-PA. D-3-PA produced a slight increase in serum free tryptophan at 50 and 75 mg/kg doses. However, the level was not significantly different from the concentration of serum free tryptophan in control animals.

The time-courses of changes in serum free tryptophan concentrations after the administrations of DL, D and L-3-PA are shown in Fig. 4b. L-3-PA (50 mg/kg) caused a significant increase of free tryptophan in the serum at 2 h after the administration, and the effect was still apparent at 96 h. This effect of L-3-PA was similar to that of DL-3-PA (100 mg/kg). The D form did not increase the concentration of free tryptophan in the serum at any of the time intervals examined.

Chronic administration (5 d) of DL (100 mg/kg/d), D (50 mg/kg/d) or L-3-PA (50 mg/kg/d) did not produce any further increase in serum free tryptophan as compared with that in animals given a single dose of DL, D or L-3-PA (data not shown).

## Discussion

In the previous[1] and present experiments, an increase in brain 5-HT concentration was observed along with a decreased TP activity in the liver and an increased level of free

NII-Electronic Library Service

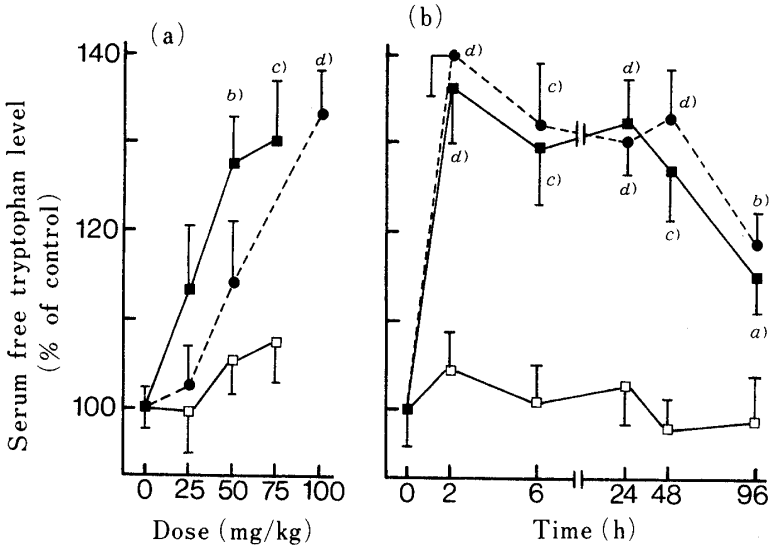


Fig. 4. Effects of DL, D and L-3-Pyridylalanine (3-PA) on the Concentration of Free Tryptophan in the Serum

Rats were injected with DL- (●---●), D- (□—□) and L-3-PA (■—■) as described in the legend to Fig. 1.
Each point represents the mean ± S.E. for 6 animals per group.
Significance of differences from controls; *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$.
Control values are as follows; Tryptophan μg/ml: (a), 2.05 ± 0.05; (b), 2.46 ± 0.10.

tryptophan in the serum upon the administration of DL-3-PA. All these effects were seen in animals administered L-3-PA. The degree of inhibition of liver TP activity after a 50 mg/kg dose of L-3-PA was not statistically different from that after a 100 mg/kg dose of DL-3-PA. Furthermore, there were no differences in the extents of increase in brain 5-HT and serum free tryptophan between a 100 mg/kg dose of DL-3-PA and a 50 mg/kg dose of L-3-PA. On the other hand, D-3-PA did not cause any changes in liver TP activity and brain 5-HT concentration even at 75 mg/kg. Serum free tryptophan concentration was also not affected by the administration of D-3-PA. These findings suggest that the changes observed in DL-3-PA-treated animals are produced by L-3-PA, and also that the peripheral conversion of D-3-PA to the L isomer does not occur in rats.

In conclusion, the present results strongly suggest that the increasing effect of DL-3-PA on brain 5-HT concentration was due mainly to the inhibition of liver TP activity by L-3-PA, and the resultant increase in the availability of circulating free tryptophan to the brain. This hypothesis is supported by the observation that L and D-3-PA hardly affected the activities of tryptophan 5-hydroxylase, 5-HTP decarboxylase and MAO, which play important roles in the synthesis and catabolism of 5-HT in the brain.

## References

1) H. Shimeno, Y. Fukumoto, A. Toda and A. Nagamatsu, *Chem. Pharm. Bull.*, **29**, 2940 (1981).
2) H. Shimeno, Y. Fukumoto, M. Fuji and A. Nagamatsu, *Chem. Pharm. Bull.*, **32**, 2353 (1984).
3) C. Nieman, R. N. Lewis and J. T. Hays, *J. Am. Chem. Soc.*, **64**, 1678 (1942).
4) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, 1961, p. 1819.

NII-Electronic Library Service