The Synthesis and Metabolism of 2,3-Dihydro-L-tryptophan and 2,3-Dihydro-5-hydroxy-DL-tryptophan*

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ABSTRACT: The catalytic reduction of various indoles related to tryptophan was accomplished in strong acid or with the 1-trifluoroacetyl derivatives in ethyl acetate. With *N,N'*-ditrifluoroacetyltryptophan methyl ester, the 2,3-dihydro derivative is formed and may be easily hydrolyzed to 2,3-dihydrotryptophan. With tritium gas, 2,3-ditritiotryptophan of high specific activity (>100 mc/mmole) was prepared. 2,3-Dihydrotryptophan, 2,3-dihydrotryptamine, and 2,3-dihydro-5-hydroxytryptophan were also prepared by catalytic reduction in the presence of strong acid. 2,3-Dihydrotryptophan and 2,3-dihydrotryptamine were metabolized in rats to 2,3-dihydroindoleacetic acid. Dehydrogenation to the parent indole with liver microsomal preparations occurred to a small extent. The 2,3-dihydroamino acids were neither substrates nor inhibitors of *aromatic amino acid decarboxylase*. Both 2,3-dihydroamino acids were *transported unchanged across the blood brain barrier*. In doses of 500 mg/kg 2,3-dihydrotryptophan produced *catatonic effects in mice.*

The pivotal role of tryptophan in various biological systems as an essential amino acid and as a precursor of the neurohormone serotonin led us to synthetize and study the biological properties of 2,3-dihydrotryptophan, 2,3-dihydrotryptamine, and the analogous 5-hydroxy compounds. The easy reduction of 1-trifluoroacetyl derivatives is an innovation which may be useful for the preparation of extremely labile indolines.

Synthetic Routes. The reduction of various indoles to indolines has been conveniently carried out using one of two methods. Thus 2,3-dihydrotryptamine, 2,3-dihydroindoleacetic acid, 2,3-dihydro-5-hydroxytryptophan, and 2,3-dihydroserotonin have been synthesized under acidic conditions by catalytic reduction of the indole with noble metal catalysts. 2,3-Dihydrotryptamine has been reported previously as a by-product of the high-pressure hydrogenation of indolylacetonitrile (Thesing and Schülde, 1952), while 2,3-dihydroindoleacetic acid has been prepared by the reduction of the ethyl (Pracejus and Köhler, 1959) and methyl esters under acidic conditions followed by saponification (Kögl and Kostermans, 1935). 2,3-Dihydroserotonin has been prepared previously by reduction under acidic conditions (Fellman *et al.*, 1962; *cf.* Smith and Utley, 1965).

We have now found that *N,N'*-ditrifluoroacetyltryptophan methyl ester is easily converted to 2,3-dihydrotryptophan by catalytic reduction with palladized charcoal in ethyl acetate followed by acid hydrolysis of the ester and *N,N'*-trifluoroacetyl groups. Photochemical reduction of tryptophan with sodium borohydride represents another route to 2,3- and 4,7-dihydro-L-tryptophan (Yonemitsu *et al.*, 1966).

The *N,N*-dibenzoyl derivative of 2,3-dihydrotryptophan and its methyl ester was also prepared. Lack of water solubility prevented the study of this compound as a substrate for the enzyme chymotrypsin. Neither the *N,N'*-dibenzoyl- nor the *N,N'*-ditrifluoroacetyl-2,3-dihydrotryptophan esters showed any indication of the presence of the two expected diastereoisomers by the criteria of thin layer, column, and vapor phase chromatography.

The 2,3-dihydroindole compounds were quite stable while the 2,3-dihydro-5-hydroxy derivatives were unstable and autoxidized readily in aqueous solution at pH 6–8 to the parent indoles in addition to other products which were not isolated. Fellman *et al.* (1962) have reported the formation of oxidation products from 2,3-dihydroserotonin and postulated a 1,6 addition of the amine side chain to the quinone imine system to form a 2,3-dihydrotrisnordehydrobufotenine. We were unable to isolate such a tricyclic dihydroindole.

Enzymatic and Metabolic Studies. A. DECARBOXYLATION. The enzyme *aromatic amino acid decarboxylase* failed to decarboxylate 2,3-dihydrotryptophan and its 5-hydroxy analog, nor were the dihydroamino acids active as competitive inhibitors. No inhibition was observed with the following compounds: isotryptophan (Kornfeld, 1951), 4,7-dihydrotryptophan, 4,5,6,7-tetrahydrotryptophan, 2-hydroxytryptophan, 6-hydroxytryptophan, 7-hydroxytryptophan, 3-aminotyrosine, α-methyl-3-methoxyphenylalanine, and phenylalanine. These and a variety of other amino acids were tested

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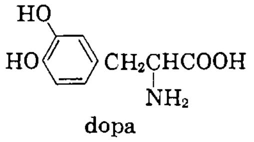
as inhibitors of the decarboxylation of both dopa[1] and 5-hydroxytryptophan. Inhibition of dopa or 5-hydroxytryptophan decarboxylation required either a 5-hydroxy group in the tryptophan series or a *m*-hydroxy group in the phenylalanine series (Table I). *threo-m*-Hydroxyphenylserine was, however, not an inhibitor. Novel compounds, such as 3-aminotyrosine and mimosine, which are structurally related to dopa did not inhibit the enzyme, while tropolonyl-γ-alanine, another structural analog of dopa, showed only slight inhibition of the enzyme. The results obtained here are not at variance with previously published data (Porter *et al.*, 1962; Lovenberg *et al.*, 1962; Weissbach *et al.*, 1960; Sourkes, 1954) and tend to substantiate the belief that *one* enzyme decarboxylates both dopa and 5-hydroxytryptophan.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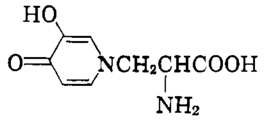
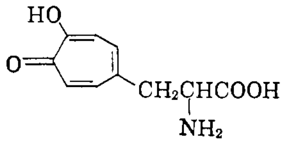
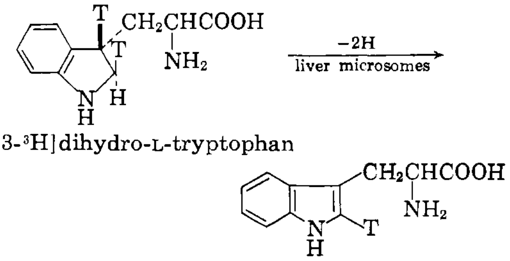

mimosine


tropolonyl-γ-alanine

B. Dehydrogenases and hydroxylases. Enzyme preparations from liver microsomes dehydrogenated 2,3-ditritiotryptophan to tryptophan to a minor extent. The ethyl ester was a slightly better substrate probably because of its greater lipid solubility. The data suggest that the dehydrogenation (Table II) of the tritiated material occurred with the abstraction of a pair of hydrogen atoms in *trans* orientation. This interpretation assumes that catalytic hydrogenation had formed a *cis*-2,3-ditritio compound.


cis-[2,3-^{3}H]dihydro-L-tryptophan

L-[2-^{3}H]tryptophan

A related dehydrogenation occurs in the conversion of succinic to fumaric acid by succinic dehydrogenase. This dehydrogenation, as shown by isotope studies, proceeds by *trans* elimination (Tchen and Van Milligan, 1960). 2,3-Dihydro-L-tryptophan was not a substrate for the tryptophan hydroxylase isolated from mast cells (W. Lovenberg, personal communication).

TABLE I: Inhibition of Aromatic Amino Acid Decarboxylase.

Inhibitor[a]	Concn	Per Cent Inhibition of [^{3}H]Dopa Decarboxylation	Per Cent Inhibition of 5-[^{14}C]-Hydroxytryptophan Decarboxylation
L-Tryptophan	2×10^{-3}	4	6
2,3-Dihydrotryptophan	2×10^{-3}	0	0
5-Hydroxytryptophan	2×10^{-3}	46	23
α-Methyl-5-hydroxytryptophan	2×10^{-3}	74	44
2,3-Dihydroxytryptophan	2×10^{-3}	0	0
5,6-Dihydroxytryptophan	2×10^{-3}	11	4
L-Dopa	2×10^{-3}	22	6
α-Methyldopa	2×10^{-3}	71	41
α-Methyl-*m*-tyrosine	2×10^{-3}	64	28
o-Tyrosine	2×10^{-3}	20	15
threo-m-Hydroxyphenylserine	2×10^{-3}	0	0
α-Methyl-2,3-dihydroxyphenylalanine	2×10^{-3}	88	83
α-Methyltyrosine	2×10^{-3}	12	5
Mimosine	2×10^{-3}	0	0
Tropolonyl-γ-alanine	1×10^{-3}	16	18
α-(3,4-Dihydroxybenzyl)-α-hydrazinopropionic acid	10^{-5}	96	97
β-(*p*-Hydroxyphenyl)-α-hydrazinopropionic acid	10^{-5}	87	90
β-Phenyl-α-hydrazinopropionic acid	10^{-5}	60	42

[a] No inhibition was observed with the following compounds: isotryptophan (Kornfeld, 1951), 4,7-dihydrotryptophan, 4,5,6,7-tetrahydrotryptophan, 2-hydroxytryptophan, 6-hydroxytryptophan, 7-hydroxytryptophan, 3-aminotyrosine, α-methyl-3-methoxyphenylalanine, and phenylalanine.

C. Metabolism *in vivo*. *In vivo*, no detectable amounts of indolic compounds were formed from 2,3-dihydro-

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[1] Abbreviation used: dopa, β-(3,4-dihydroxyphenyl)-L-alanine.

TABLE II: The Dehydrogenation of 2,3-[2,3-^{3}H]Dihydrotryptophan (Ethyl Ester) by Rabbit Liver Homogenates and Subcellular Fractions. Assay as Described in the Text.

	Tritiated Water Formed from			
	2,3-[2,3-^{3}H]Dihydrotryptophan		2,3-[2,3-^{3}H]Dihydrotryptophan Ethyl Ester	
Source of Dehydrogenase Act.	Total (cpm)	mμ-moles/hr g of Tissue	Total (cpm)	mμ-moles/hr g of Tissue
Boiled tissue homogenate	440	–	740	–
Tissue homogenate	2100	0.3	–	–
Microsomal fraction	2700	0.4	4200	0.8
Mitochondrial fraction	900	0.07	–	–
Soluble supernatant fraction	640	0.03	–	–

TABLE III: Paper Chromatography of Various Potential Metabolites of 2,3-Dihydrotryptophan.[a]

	Solvent		
Compound	a	b	c
2,3-Dihydrotryptophan	0.33	0.42	0.33
Tryptophan	0.46	0.43	0.37
2,3-Dihydrotryptamine	0.67	0.66	0.85
Tryptamine	0.87	0.72	0.96
2,3-Dihydroindoleacetic acid	0.78	0.92	0.60
Indoleacetic acid	0.95	0.90	0.55
Metabolite A (major)	0.76	0.89	0.62
Metabolite B (minor)	0.83	0.89	0.41

[a] Solvents: (a) butanol–acetic acid–water (4:1:1); (b) butanol–acetic acid–pyridine–water (4:1:1:2); (c) isopropyl alcohol–ammonia–water (8:1:1) on Whatman No. 1. Metabolite A also migrated with 2,3-dihydroindole acetic acid in three other solvent systems (see Figure 1).

tryptophan or 2,3-dihydrotryptamine. The major metabolite of the amino acid was 2,3-dihydroindoleacetic acid (Figure 1). In view of the lack of activity of 2,3-dihydrotryptophan as a substrate for aromatic amino acid decarboxylase, 2,3-dihydroindole-3-acetic acid must be formed through decarboxylation of 2,3-dihydroindolepyruvic acid which would result from transamination of the amino acid. The formation of dihydroindoleacetic acid was not blocked by pretreatment of the animal with the monoamine oxidase inhibitor, marsilid. Table III presents chromatographic data on potential metabolites of 2,3-dihydrotryptophan and the two radioactive metabolites found in urine, one of which was identified as 2,3-dihydroindoleacetic acid.

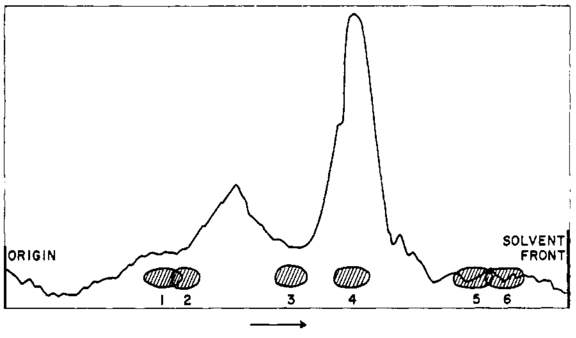

FIGURE 1: Radioactivity scan of a paper chromatogram of urinary metabolites from 2,3-[2,3-^{3}H]dihydrotryptophan in rat (Whatman No. 1, isopropyl alcohol–ammonia–water, 8:1:1). Reference compounds: (1) 2,3-dihydrotryptophan, (2) tryptophan, (3) indoleacetic acid, (4) 2,3-dihydroindoleacetic acid, (5) 2,3-dihydrotryptamine, and (6) tryptamine.

2,3-Dihydrotryptamine was also converted *in vivo* to 2,3-dihydroindoleacetic acid but this conversion was partially blocked by marsilid. Fellman *et al.* (1962) have reported that 2,3-dihydroserotonin is oxidized by monoamine oxidase.

D. ACTIVE TRANSPORT. The amino acid, 2,3-dihydrotryptophan, was readily transported unchanged into brain tissue as shown in Table IV. Other reduced tryptophans were not transported as readily into brain tissue and were not identified from this organ. 2,3-Dihydro-5-hydroxytryptophan was concentrated both in brain and intestine (Table IV and Experimental Section). Some conversion of 2,3-dihydro-5-hydroxytryptophan to 5-hydroxytryptophan was observed in these studies, but it is not known whether this is the result of nonenzymatic autoxidation or of enzymatic dehydrogenation.

Administration of 500 mg/kg of 2,3-dihydrotryptophan to mice led to markedly reduced activity similar to a catatonic state. This effect was not observed with 2,3-dihydro-5-hydroxytryptophan. Experimental catatonia is of diagnostic value for agents acting on the central nervous system and has been implied recently in connection with mental disorders (Brown *et al.*, 1965).

Experimental Section

2,3-Dihydro-L-tryptophan. A. FROM L-TRYPTOPHAN. A solution of 2.08 g of L-tryptophan in 35 ml of 1.0 N hydrochloric acid containing 400 mg of 10% palladized charcoal was shaken under hydrogen at 1 atm

TABLE IV: Uptake of Radioactive Tryptophans into Mouse Tissues.[a]

Compound	Time	Blood (mμc/g)	Muscle (mμc/g)	Brain (mμc/g)	Ratio Brain: Plasma
DL-[^{3}H]Tryptophan (1 mg, 10 mμc)	15	0.63	0.32	0.11	0.2
	30	0.30	0.23	0.19	0.6
	90	0.22	0.20	0.20	0.9
	180	0.12	0.13	0.17	1.4
2,3-[^{3}H]Dihydrotryptophan (1 mg, 10 mμc)	15	1.20	0.54	0.36	0.3
	30	0.66	0.66	0.44	0.7
	90	0.36	0.37	0.42	1.2
	180	0.29	0.32	0.39	1.3
4,5,6,7-[^{3}H]Tetrahydro-L-tryptophan (1 mg, 10 mμc)	15	0.53	0.25	0.08	0.2
	30	0.33	0.20	0.12	0.4
	90	0.19	0.15	0.14	0.7
	180	0.15	0.12	0.14	0.9
4,7-[^{3}H]Dihydro-L-tryptophan (1 mg, 10 mμc)	15	0.41	0.38	0.15	0.4
	30	0.32	0.29	0.19	0.6
	90	0.20	0.17	0.16	0.8
	180	0.13	0.10	0.12	0.9
[^{14}C]5-Hydroxytryptophan (1 mg, 2 mμc)	15	0.20	0.12	0.04	0.2
	30	0.09	0.08	0.05	0.6
	90	0.08	0.06	0.06	0.8
	180	0.04	0.02	0.06	1.5
2,3[^{14}C]Dihydro-5-hydroxytryptophan (1 mg, 2 mμc)	15	0.25	0.12	0.08	0.3
	30	0.09	0.07	0.06	0.7
	90	0.04	0.04	0.05	1.2
	180	0.03	0.02	0.03	1.0

[a] Assay as described in the text. Dose is per 20-g mouse.

for 30 hr during which time 1.2 equiv of hydrogen gas was absorbed. The catalyst was removed by filtration, and the filtrate was made alkaline with aqueous ammonia and evaporated to dryness *in vacuo*. Recrystallization from water yielded 1.04 g (50%) of 2,3-dihydro-L-tryptophan, mp 275° dec, $[\alpha]_D^{20}$ $-10.7 \pm 2.0°$ (*c* 0281, 1.0 N NaOH). The mass spectrum confirmed the purity and identity. *Anal.* Calcd for parent peak for $C_{11}H_{14}N_2O_2$: 206.106. Found: 206.108. Calcd for base peak C_8H_8N: 118.070. Found: 118.068. Calcd for an intense peak $C_9H_{10}N$: 132.081. Found: 132.083. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.05; H, 6.84; N, 13.58. Found: C, 63.75; H, 6.69; N, 13.31.

B. FROM *N,N'*-DITRIFLUOROACETYL-2,3-DIHYDRO-L-TRYPTOPHAN METHYL ESTER: A solution of *N,N'*-ditrifluoroacetyl-L-tryptophan methyl ester (2.08 g) (Makisumi and Saroff, 1965) in ethyl acetate (50 ml) containing 1 g of 10% palladized charcoal was shaken under hydrogen at 1 atm for 19 hr. Gas chromatography on a column (3 ft) of 3% SE30 on Chrom WAW at 175° showed three peaks at 3.3 (small, unknown), 4.0 (large, *N,N'*-ditrifluoroacetyl-2,3-dihydro-L-tryptophan methyl ester), and 4.8 min (small, mono-*N*-trifluoroacetyl-L-tryptophan methyl ester). The solution was filtered through a column of Hyflo Super Cel and evaporated. The residue was chromatographed on silica gel (Merck, 0.05–0.20 mm) in benzene–ethyl acetate (17:3). The fractions (25 ml) were collected and assayed by gas chromatography. The product was located in fractions 12–15. After evaporation, the residual oil was purified by molecular distillation (0.1 mm) at 190°. The colorless oil (1.26 g) set to a rigid glass but could not be crystallized. Gas chromatography on a column (6 ft) of 3% neopentylglycol succinate on Gaschrom Z at 250° gave a single symmetrical peak at 17.6 min; ν_{max}^{KBr} 3450 (NH), 1730 (C=O), and 1700 cm^{-1} (amide); $\lambda_{max}^{CH_3CN}$ 257 mμ (ϵ 10,700). *Anal.* Calcd for $C_{16}H_{12}F_6N_2O_4$: C, 46.61; H, 3.42; F, 27.65; N, 6.80. Found: C, 46.45; H, 3.61; F, 26.7; N, 6.78.

The *N,N'*-ditrifluoroacetyl-2,3-dihydro-L-tryptophan methyl ester (597 mg) was dissolved in 5 ml of acetic acid and 7 ml of concentrated hydrochloric acid was added. The solution was heated under reflux for 1.5 hr and evaporated *in vacuo*, and the residue was taken up in 10 ml of water, brought onto a column of Bio-Rad AG 50W-Xi, and washed with water until the washings were neutral. The product was eluted with 3.0 N ammonium hydroxide. Evaporation *in vacuo* and recrystallization from water gave colorless plates (291 mg) of 2,3-dihydrotryptophan identical in all respects with the material prepared by method A.

*2,3-[2,3-^{3}H]Dihydro-*L*-tryptophan.* *N,N'*-Ditrifluoroacetyl-L-tryptophan methyl ester (10 mg) was reduced with tritium gas over 10% palladized charcoal (10 mg) in 5 ml of ethyl acetate (New England Nuclear Corp.). To an aliquot of the ethyl acetate solution, equivalent to 0.4 mg of the amino acid, was added 3.6 mg of carrier *N,N'*-ditrifluoroacetyl-2,3-dihydro-L-tryptophan methyl ester. The solution was evaporated *in vacuo*, and the residue, in 1 ml of acetic acid, was mixed with 1 ml of concentrated hydrochloric acid and heated in a sealed tube at 115° for 6 hr. The solution was evaporated *in vacuo* and reevaporated twice with water (3 ml). The residue which was dissolved in 2 ml of water had a specific activity of 100 mc/mmole. Final purification was carried out by preparative paper chromatography in butanol–acetic acid–water (4:1:1).

2,3-Dihydrotryptophan Ethyl Ester. 2,3-Dihydrotryptophan (100 mg) was dissolved in 20 ml of ethanol previously saturated with hydrochloric acid. After 18 hr the solution was evaporated *in vacuo*, taken up in 5 ml of water, and neutralized with sodium bicarbonate. Extraction with ethyl acetate, drying (Na_2SO_4), and evaporation *in vacuo* afforded an oil. This was dissolved in dilute hydrochloric acid, evaporated to dryness *in vacuo*, and recrystallized from ethanol–ethyl acetate to yield 60 mg of a tan hygroscopic powder, mp 170–174° dec, ν_{max}^{KBr} 1740 cm^{-1} (ester C═O). *Anal.* Calcd parent peak (mass spectrum) for $C_{13}H_{18}N_2O_2$: 234.137. Found: 234.138. The base peak was at 118 corresponding to loss of $CH_2CH(NH_2)COOC_2H_5$.

*2,3-[2,3-^{3}H]Dihydro-*L*-tryptophan ethyl ester* was prepared in the same manner using the 2,3-[2,3-^{3}H]-dihydro-L-tryptophan described above. Paper electrophoresis at pH 1.9 revealed only one peak corresponding to 2,3-dihydrotryptophan ethyl ester.

*N,N'-Dibenzoyl-2,3-dihydro-*L*-tryptophan.* To a solution of 0.103 g of 2,3-dihydro-L-tryptophan in 5 ml of 0.1 N sodium hydroxide were added at 5° and pH 8–9 with vigorous stirring 0.16 g of benzoyl chloride and 10 ml of 0.1 N sodium hydroxide. The mixture was stirred for 15 min and extracted with ether (three 10-ml portions). The aqueous layer was acidified with 0.1 N hydrochloric acid and extracted with chloroform. The chloroform layer was washed with water and dried (Na_2SO_4). The solution was evaporated and the residue was triturated with ether and recrystallized from 50% aqueous methanol to yield 0.165 g (80%), mp 200–201°, of *N,N'*-dibenzoyl-2,3-dihydrotryptophan, $[\alpha]_D^{20}$ −27.7° (*c* 1.146, $CHCl_3$), ν_{max}^{KBr} 1600 (COOH) and 1640 cm^{-1} (amide C═O). *Anal.* Calcd for $C_{25}H_{22}N_2O_4$: C, 72.44; H, 5.35; N, 6.77. Found: C, 72.52; H, 5.40; N, 6.55.

N,N'-Dibenzoyl-2,3-dihydrotryptophan Methyl Ester. To a solution of *N,N'*-dibenzoyl-2,3-dihydrotryptophan (0.14 g) in tetrahydrofuran (3 ml) was added 2 ml of ethereal diazomethane. After 30 min the excess diazomethane was decomposed with acetic acid and then the solution was evaporated to dryness. The residue was recrystallized from methanol to yield 0.14 g (93%) of *N,N'*-dibenzoyl-2,3-dihydrotryptophan methyl ester, mp 201–202°, insoluble in water, ν_{max}^{KBr} 1750 (ester C═O) and 1645 cm^{-1} (amide C═O). *Anal.* Calcd for $C_{26}H_{24}N_2O_4$: C, 72.88; H, 5.65; N, 6.54. Found: C, 72.96; H, 5.43; N, 6.50.

2,3-Dihydrotryptamine. A solution of 0.66 g of tryptamine hydrochloride in 20 ml of 1.5 N hydrochloric acid was reduced with hydrogen at 1 atm for 30 hr in the presence of 100 mg of 10% palladized charcoal during which time 1.1 equiv of hydrogen was taken up. Evaporation *in vacuo* and repeated recrystallization from a mixture of ethanol and ethyl acetate afforded 280 mg (42%) of colorless crystals of 2,3-dihydrotryptamine hydrochloride, mp 190–190.5°. *Anal.* Calcd for $C_{10}H_{15}ClN_2$: C, 60.45; H, 7.61; Cl, 17.85; N, 14.11. Found: C, 60.60; H, 7.48; Cl, 18.00; N, 13.98.

Dipicrate showed: mp 178–180°, lit. (Thesing and Schülde, 1952) mp 175–176°. *Anal.* Calcd for $C_{22}H_{20}N_8O_{14}$: N, 18.06. Found: N, 18.31.

2,3-Dihydroindoleacetic Acid. To a solution of 0.88 g of indoleacetic acid in 20 ml of acetic acid was added 20 ml of 2.0 N hydrochloric acid. In the presence of 400 mg of 10% palladized charcoal the solution took up 0.9 equiv of hydrogen at 1 atm in the course of 48 hr. Paper chromatography of the filtered reaction mixture showed one major spot with typical indoline color reactions (yellow with Ehrlich's reagent). Evaporation afforded 0.6 g (57%) of a colorless oil which was converted to the picrate of 2,3-dihydroindoleacetic acid, mp 166–167°, lit. (Kögl and Kostermans, 1935) mp 168°. *Anal.* Calcd for $C_{16}H_{14}N_4O_9$: N, 13.79. Found: N, 13.95.

2,3-Dihydroserotonin. A solution of serotonin creatinine sulfate (310 mg) in 15 ml of acetic acid and 1.5 ml of concentrated hydrochloric acid was reduced with hydrogen at 1 atm in the presence of 150 mg of platinum oxide for 6 hr, during which time 1.0 equiv of hydrogen was taken up. The mixture was filtered and evaporated to dryness *in vacuo*. Attempted purification of this pinkish powder was unsuccessful. The reduction product gave a single spot on paper chromatography (Whatman No. 1) in butanol–acetic acid–water (3:1:1), R_F 0.28, and a yellow Ehrlich reaction. Serotonin (R_F 0.42, Ehrlich reaction purple) was not present. After autoxidation overnight at pH 8 paper chromatography revealed serotonin and two new compounds, one having R_F 0.33 and giving a yellow Ehrlich reaction, the other having R_F 0.20 and giving a red-yellow Ehrlich reaction (see Fellman *et al.*, 1962). The mass spectrum of 2,3-dihydroserotonin was consistent with the structure $C_{10}H_{14}N_2O$. *Anal.* Calcd for $C_{10}H_{14}N_2O$: 178.111. Found: 178.109 (with a base peak at 134 corresponding to benzylic cleavage).

2,3-Dihydro-5-hydroxytryptophan. A solution of DL-5-hydroxytryptophan (219 mg) in 5 ml of acetic acid and 1.5 ml of concentrated hydrochloric acid on reduction with hydrogen at 1 atm in the presence of 150 mg of platinum oxide for 2.5 hr took up 1.1 equiv of hydrogen. The solution was filtered, treated with Norit, and evaporated *in vacuo*. The residue was recrystallized from ethanol–ethyl acetate to yield 140 mg of the hydrochloride of 2,3-dihydro-5-hydroxytryptophan. No unreduced 5-hydroxytryptophan was detected in this sample by the criteria of paper chromatography (butanol–acetic acid–water, 3:1:1) and paper electrophoresis at pH 1.9. Autoxidation at pH 7–8 led back to 5-hydroxytryptophan. The mass spectrum was consistent with the structure, having a parent peak at 222 and a base peak corresponding to benzylic cleavage at 134.

2,3-[3′-^{14}C]Dihydro-5-hydroxytryptophan. A solution of DL-5-[3′-^{14}C]hydroxytryptophan (22 mg, 0.02 mc) in 5 ml of acetic acid containing 0.5 ml of concentrated hydrochloric acid was reduced in the presence of 15 mg of platinum oxide for 4 hr. The material was first purified as described above and then by preparative paper electrophoresis in pH 1.9 buffer. Only one radioactive product was presenti nt his final material, as judged by paper chromatography and paper electrophoresis.

Enzymatic Decarboxylation of Dihydrotryptophans. The enzyme *aromatic amino acid decarboxylase* from guinea pig kidney was purified by differential centrifugation and ammonium sulfate precipitation (Clark, *et al.*, 1954). Incubations were carried out in a total volume of 1 ml containing 0.2 ml of 0.5 N phosphate buffer, pH 7.4, 2 × 10^{-5} M harmaline, 2 × 10^{-4} M pyridoxal phosphate, and 0.4 ml of enzyme preparation containing approximately 10 mg of protein, and substrate in a concentration of 10^{-3}–10^{-5} M. The mixture was preincubated for 5 min before the addition of the substrate. Incubation was then continued for 30 min. The incubations were stopped by the addition of 1.0 N acid and lyophilized, and aliquots were subjected to paper electrophoresis at pH 1.9. Under these conditions the conversions of tryptophan to tryptamine and 5-hydroxytryptophan to serotonin were readily detected. With the tritium-labeled amino acids neither formation of radioactive 2,3-dihydrotryptamine nor 2,3-dihydroserotonin from their parent amino acids could be detected, nor were amines detected from 4,7-[2′,3′-^{3}H]dihydrotryptophan or 4,5,6,7-[2′,3′-^{3}H]tetrahydrotryptophan prepared by the method of Yonemitsu *et al.* (1966).

Studies on Inhibition of the Decarboxylation of [^{3}H]Dopa and 5-[3′-^{14}C]Hydroxytryptophan by 2,3-Dihydrotryptophans and Other Aromatic Amino Acids. Incubations were carried out as described above with substrate and inhibitor added after 5-min preincubation. Incubation was continued for 30 min. With [^{3}H]dopa as substrate the concentration was 1 × 10^{-4} M (150,000 cpm). The reaction was assayed using a modification of the procedure of Lovenberg *et al.* (1962) in which the incubation was stopped by heating, and the radioactivity of the dopamine passing through the ion-exchange column was measured instead of the fluorescence. Zero-time and boiled enzyme experiments gave blanks of 100–300 cpm for a 1-ml aliquot of the acetic acid effluent (5 ml). Without inhibitor values of 2800–3000 cpm were obtained. For 5-[3′-^{14}C]hydroxytryptophan (1 × 10^{-4} M, 200,000 cpm) a modification of the extraction procedure of Snyder and Axelrod (1964) was employed in which the reaction is stopped by the addition of 0.8 ml of borate buffer, pH 10, and the product was extracted into 10 ml of isoamyl alcohol–toluene (8:1). This organic layer is reextracted with 1 ml of 1.0 N HCl of which 0.5 ml is used for counting. For zero-time and boiled enzyme experiments background readings averaged 300–500 cpm; incubations gave reading of 2200–2400 cpm. Table I summarizes the inhibition data.

Dehydrogenation of 2,3-[2,3-^{3}H]Dihydro-L-tryptophan with Liver Microsomes. Incubations were carried out with preparations from rabbit liver homogenized with isotonic KCl (1:3). Incubation mixtures contained 0.3 ml of tissue preparation, 0.2 ml of 5.0 M phosphate buffer, pH 7.40, 0.6 μmole of niacinamide–adenine dinucleotide phosphate, 1.5 μmoles of glucose 6-phosphate, and 1 mμmole of either 2,3-[2,3-^{3}H]-dihydro-L-tryptophan (60,000 cpm) or 2,3-[2,3-^{3}H]-dihydro-L-tryptophan ethyl ester (42,000 cpm) in a total volume of 0.8 ml. After 1-hr incubation the mixture was heated for 1 min on a boiling water bath and centrifuged. The supernatant was poured onto a column of Dowex 50 ion-exchange resin buffered at pH 6.5. The column was washed with 1 ml of water. A 1-ml aliquot of the effluent and wash was counted by liquid scintillation to determine the amount of ^3H_2O formed by dehydrogenation of the 2,3-dihydroindole. That the radioactivity represented only water was determined by evaporation which resulted in complete loss of radioactivity. The results are presented in Table II. In the case of 2,3-dihydrotryptophan ethyl ester a tenfold larger experiment was carried out. One-half was assayed for ^3H_2O and the other portion was lyophilized and, after addition of carrier 2,3-dihydrotryptophan ethyl ester and tryptophan ethyl ester, subjected to preparative paper electrophoresis at pH 1.9. The amount of radioactivity in the tryptophan ethyl ester fraction was compared to the amount detected in the water formed. They were: ^3H_2O, 21,000 cpm; [2-^{3}H]tryptophan ethyl ester, 14,000 cpm. *cis* elimination should have resulted in no activity in the tryptophan ethyl ester, while *trans* elimination would lead to equal activities in water and tryptophan ethyl ester.

Metabolism of 2,3-[2,3-^{3}H]Dihydro-L-tryptophan. Rats (Sprague–Dawley, 250 g) were given 2.5 mg (0.5 mc) of 2,3-dihydrotryptophan intraperitoneally. Urine was collected and cooled at 0° during 16 hr. After lyophilization the residue was dissolved in methanol for paper chromatography. Other rats were given 120 mg/kg of marsilid intraperitoneally 8 and 1 hr before the administration of L-[2,3-^{3}H]tryptophan. A total of 0.12 (24%) and 0.09 mc (18%) was excreted within 16 hr by the first group (two rats) and the marsilid-treated rats (two), respectively. The metabolites in the urine were not significantly different in these two groups. The major metabolite was identified as 2,3-dihydroindoleacetic acid as shown in Table III and Figure 1. The minor metabolite was not identified. No indoleacetic acid was detected. Similarly, rats given 6 mg of 2,3-dihydrotryptamine intraperitoneally had large amounts of 2,3-dihydroindoleacetic acid and smaller amounts of other nonindolic metabolites. Treatment with marsilid decreased the formation of 2,3-dihydroindoleacetic acid and increased the amount of 2,3-dihydrotryptamine excreted.

Transport of Dihydrotryptophan into Brain. The labeled dihydrotryptophan was administered intravenously to mice averaging 14–18 g. The animals were killed at intervals and the blood, muscle, brain, and certain other tissues were homogenized with ten volumes of 0.4 N perchloric acid. The radioactivity of an aliquot was determined. The results are presented in Table IV.

In the case of 2,3-[2,3-^{3}H]dihydro-L-tryptophan, the elution of the radioactivity in brain homogenates coincided with elution of carrier 2,3-dihydrotryptophan from a column of Dowex 1-X8, acetate form, eluent 0.2 N acetic acid. With 4,7-[2′,3′-^{3}H]dihydro- and 4,5,6,7-[2′,3′-^{3}H]tetrahydrotryptophan the radioactive compound in brain could not be identified. For 2,3-[3′-^{14}C]dihydro-5-hydroxytryptophan, the brain homogenate and intestine homogenate were neutralized and passed through a short column of Dowex 1-X8 in the acetate form. Elution with 0.2 N acetic acid and evaporation *in vacuo* yielded a concentrated solution which contained in each case principally 2,3-dihydro-5-hydroxytryptophan on the basis of paper electrophoretic analysis at pH 1.9.

After intraperitoneal administration of 500 mg/kg of 2,3-dihydrotryptophan, mice exhibited markedly reduced activity which approached a catatonic state. Although capable of locomotion and still responsive to stimuli, such as noise and prodding, the mice retained unusual positions in which they were placed. This condition lasted for several hours and was not accompanied by sedation or other gross pharmacological effects. The animals completely recovered after 16 hr. The administration of the same dosage of 2,3-dihydro-5-hydroxytryptophan did not elicit this reaction, but in contrast caused a slight degree of hyperactivity in the animals.

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