PREPARATION OF STEREOSPECIFICALLY α- AND β-TRITIATED TRYPTAMINE AND THE STEREOCHEMISTRY OF AROMATIC L-AMINO ACID DECARBOXYLASE

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For studies on some aspects of the biosynthesis of indole alkaloids (1, 2), we required the four isomers of tryptamine tritiated stereospecifically at the α- and the β-position of the side chain. Enzymatic decarboxylation of tryptophan seemed the most convenient principal approach, and aromatic L-amino acid decarboxylase (E.C.4.1.1.28) was chosen as the enzyme to catalyze this conversion. This pyridoxal phosphate-containing enzyme is widely distributed in mammalian tissues and is responsible for the production of the neuroactive amines dopamine and serotonin from 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan, respectively (3,4). The enzyme from hog kidney also decarboxylates L-tryptophan, L-tyrosine and L-phenylalanine at appreciable rates (3).

RESULTS AND DISCUSSION

In order to prepare (2**R**)- and (2**S**)-[2-^{3}H]tryptamine, (2**S**,3**S**)- and (2**S**,3**R**)-[3-^{3}H]serine, available from earlier work (5), were converted into (2**S**,3**R**)- and (2**S**,3**S**)-[3-^{3}H]tryptophan with tryptophan synthase as previously described (6). These tryptophan samples (10μCi/100 nmoles) were then decarboxylated with aromatic L-amino acid decarboxylase partially purified from hog kidney by a modification of the procedure of Christenson *et al* (3). Since the decarboxylation does not involve any bond breakage or formation at the stereospecifically labeled center, the configuration of the two resulting tryptamine samples at C–2 must be the same as the known (6) configuration of the tryptophan samples from which they were derived. The tryptophan samples were shown (6) to be chirally pure at C–3 within the limits of detection, and the same must thus be true for the tryptamine samples derived from them.

To obtain the two isomers of tryptamine tritiated stereospecifically at C–1 of the side chain, we made use of the anticipated stereospecificity of the aromatic L-amino acid decarboxylase. All pyridoxal phosphate-dependent amino acid α-decarboxylases studied to date operate by replacing the carboxyl group stereospecifically with a solvent proton without labilizing the α-hydrogen of the amino acid, and the steric course of the replacement in all cases reported so far is retention (7–9). In fact, while this work was in progress, Battersby *et al.* (13) reported that the aromatic L-amino acid decarboxylase decarboxylates (2**S**)-[2-^{3}H]tyrosine in a retention mode to give (1**S**)-[1-^{3}H]tyramine. We therefore incubated non-labeled L-tryptophan with the decarboxylase in the presence of tritiated water to generate a chirally tritiated tryptamine of, presumably, 1**R** configuration. The opposite enantiomer was generated by decarboxylation of **S**-[2-^{3}H] tryptophan, which was prepared either by incubation of L-tryptophan with tryptophan synthase in tritiated water (6) or by N-chloroacetylation of **R**,**S**-[2-^{3}H]tryptophan (10) and resolution with carboxypeptidase (11).

To verify the configurations of the two enantiomers of the 1-tritiated tryptamine and to assess their chiral purity, we incubated these samples, mixed with [^{14}C]tryptamine, with monoamine oxidase (MAO) from rat liver mitochondria. This enzyme ex-

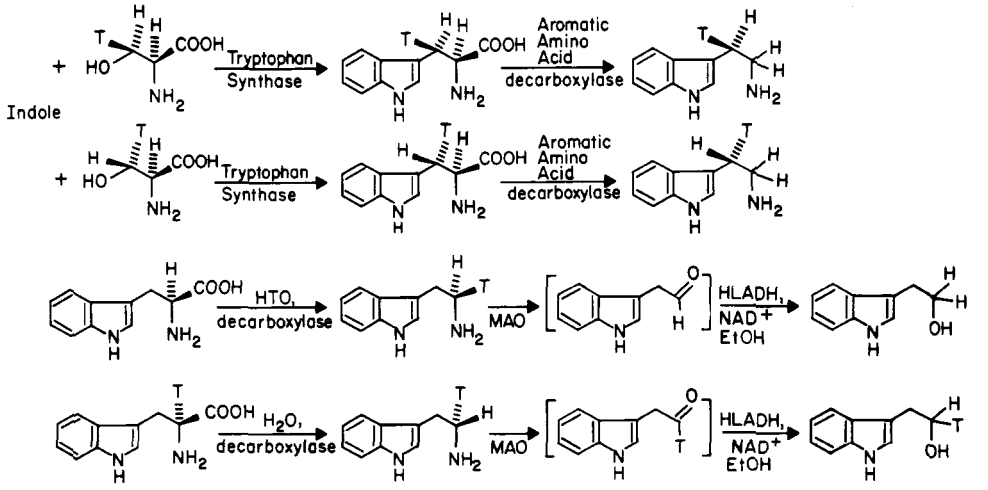
TABLE 1. Stereochemical analysis of [1-^{3}H]tryptamine produced by decarboxylation of tryptophan

Experiment	Substrate for decarboxylation	Reaction medium	Tryptamine $^3H/^{14}C$ Ratio	Tryptophol $^3H/^{14}C$ Ratio	Configuration of [1-^{3}H] tryptamine
1	(2*S*)-Tryptophan	[^{3}H]H_2O	4.88	0.56	*R*
2	(2*S*)-Tryptophan	[^{3}H]H_2O	16.88	1.45	*R*
3	(2*S*)-[2-^{3}H] Tryptophan[a]	H_2O	6.67	7.70	*S*
4	(2*S*)-[2-^{3}H] Tryptophan[a]	H_2O	7.66	9.18	*S*
5	(2*S*)-[2-^{3}H] Tryptophan[b]	H_2O	11.92	14.37	*S*

[a]Produced by incubation with tryptophan synthetase in [^{3}H]H_2O.
[b]Produced by resolution with carboxypeptidase.

hibits stereospecificity for removal of the **pro-R** hydrogen from a $-CH_2-NH_2$ group (12, 13). The resulting indoleacetaldehyde, a very unstable compound, was reduced *in situ* with liver alcohol dehydrogenase and excess ethanol to give tryptophol. The results of several analyses are listed in table 1. It is obvious from the data that decarboxylation of L-tryptophan in tritiated water has produced [1-^{3}H]tryptamine of 1**R** configuration (expts. 1 and 2); hence the decarboxylase indeed operates in a retention mode with this substrate as well. The chiral purity of these tryptamine samples is about 90%; the slight scrambling of tritium between the two heterotopic positions may be due to contamination of the decarboxylase with some other pyridoxal phosphate enzymes which catalyze slow exchange of the α-hydrogen of tryptophan with solvent protons. The results of experiments 3–5 (table 1) indicate complete retention of tritium in the MAO reaction, congruent with the notion that the [1-^{3}H]tryptamine obtained by decarboxylation of (2**S**)-[2-^{3}H] tryptophan has 1**S** configuration. There is, in fact, a slight increase in the $^3H/^{14}C$ ratio upon conversion of tryptamine to tryptophol; this may be due to further oxidation of indoleacetaldehyde by MAO, with a large kinetic isotope effect, as a side reaction competing with reduction to the alcohol.

In summary, the work described provides viable routes for the preparation of all four isomers of stereospecifically side-chain tritiated tryptamine as shown in scheme I. In addition it verifies that, as expected, the decarboxylation of L-tryptophan catalyzed by the aromatic L-amino acid oxidase from hog kidney proceeds in a retention mode.



SCHEME 1. Preparation and analysis of stereospecifically tritiated tryptamine samples.

EXPERIMENTAL

MATERIALS.—L-Tryptophan, NAD^+, pyridoxal phosphate and horse liver alcohol dehydrogenase (HLADH) were purchased from Sigma, L-[3-^{14}C]tryptophan (56 mCi/mmole) from Amersham/Searle, and [2-^{14}C] tryptamine (51 mCi/mmole), [2-^{3}H]dopa (50 mCi/mmole) and [^{3}H] H_2O (100 mCi/ml) from New England Nuclear. (2**S**,3**R**)- and (2**S**,3**S**)-[3-^{3}H] serine, available from previous work (5) were converted into tryptophan with tryptophan synthase as described earlier (6). (2**S**)-[2-^{3}H]Tryptophan was available from earlier work (11) or was prepared by exchange of the non-labeled compound with [^{3}H]H_2O and tryptophan synthase (6). A homogeneous preparation of tryptophan synthase $\alpha_2\beta_2$ protein (50 mg/ml) was obtained as a gift from Dr. Edith W. Miles, National Institutes of Health. Aromatic L-amino acid decarboxylase was partially purified from hog kidney by an abbreviated version of the published procedure (3), which included the streptomycin sulfate and ammonium sulfate precipitation and the DEAE-Sephadex column separation steps. Fractions containing tryptophan decarboxylase activity were precipitated with 2 volumes of saturated $(NH_4)_2SO_4$ solution, centrifuged, and stored as the precipitate at −20° for up to 2 months without loss of activity. Immediately before use, the enzyme was dissolved in 0.08M Tris buffer, pH 8.0 containing 10^{-5}M pyridoxal phosphate and 10^{-4}M dithiotheitol and dialyzed against 2 changes of the same buffer. The resulting enzyme solution contained about 4 mg protein/ml; two ml of the solution under the assay conditions will decarboxylate 200 nmoles L-tryptophan/hr. A crude preparation of MAO from rat liver was obtained according to a published procedure (14); the enzyme was assayed by following the oxidation of benzylamine at 250 nm (15).

METHODS.—Preparative and analytical thin layer chromatography employed precoated silica gel plates (F 254; 2.0 and 0.25 mm) (systems B and C) and cellulose plates (F 254; 0.1 mm) (system A) obtained from Merck. The following solvent systems were used: System A, 2-propanol/water/acetic acid, 70:25:5, tryptophan Rf 0.55, tryptamine Rf 0.85; System B, ethyl acetate/chloroform/methanol, 60:30:10, tryptamine Rf 0.10, tryptophol Rf 0.75; System C, ethanol/water, 70:30, tryptamine Rf 0.20. Methods of visualization included uv light, ninhydrin and iodine vapor. Liquid scintillation counting was performed on a Beckman LS-7500 system with Aquasol (New England Nuclear) as the scintillation medium. All samples were counted to a statistical error of at least 2%. Counting efficiencies and the spillover of ^{14}C into the tritium channel were determined by internal standardization with [^{14}C]- and [^{3}H]toluene. Radioactivity on chromatograms was located with a Packard 7201 radiochromatogram scanner.

DECARBOXYLATION OF L-TRYPTOPHAN.—In a typical incubation, 0.5–2.0 mg of tritiated L-tryptophan was incubated with 2 ml of the decarboxylase solution for 1 hour at 37°; more enzyme (2 ml) was added and the incubation continued for another hour. The reaction was stopped by the addition of ten drops of 5N NaOH, and the tritiated tryptamine was isolated by extraction with 2 x 5 ml benzene. The benzene layer was washed with an equal volume of 0.1N NaOH, concentrated to a smaller volume, and the tryptamine was then extracted into 1 ml 0.25 N HCl. The radiochemical purity of the tryptamine was checked by chromatography in systems A and C. For the decarboxylation in tritiated water, the decarboxylase solution was lyophilized and to the residue were added 0.5 mg of L-tryptophan and 2 ml tritiated water (100 mCi/ml). After the incubation the tritiated water was removed by lyophilization, the residue was dissolved in water, and the enzyme was inactivated by heat (2 min. 60°). Another lyophilization removed the bulk of the exchangeable tritium, following which the tryptamine was isolated as described above.

OXIDATION OF TRITIATED TRYPTAMINES WITH MAO.—Prior to incubation with MAO, the tritiated tryptamine samples were combined with [2-^{14}C]tryptamine and constancy of the ^{3}H/^{14}C ratio was ascertained by repeated thin layer chromatography in systems A and C. The suspension of rat liver mitochondria (14) (2 ml) was incubated at 32° with 2 mg tryptamine, 10 mg NAD^+, 2 mg HLAD, and 100 μl ethanol. After 24 hours the reaction mixture was acidified to pH 1 with 2.5N HCl and centrifuged to remove the precipitated protein. Tryptophol was extracted into ether, concentrated under nitrogen and chromatographed in system B. The section of silica gel corresponding to the position of reference tryptophol was removed and extracted with methanol for the determination of the ^{3}H/^{14}C ratio.

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