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2 December 1974

Tryptoline Formation by a Preparation from Brain with 5-Methyltetrahydrofolic Acid and Tryptamine

Abstract. *An enzymatic preparation from human brain converts tryptamine to tryptoline (9H-1,2,3,4-tetrahydropyrido[3,4-b]indole) in the presence of 5-methyltetrahydrofolic acid. Similarly, N-methyltryptamine and 5-hydroxytryptamine yield 1-methyltryptoline and 5-hydroxytryptoline, respectively. Neither in vitro nor in vivo formation of these compounds by human tissues has been described.*

Administration of the amino acids tryptophan and methionine to schizophrenic patients exacerbates their illness, giving rise to suggestions that endogenous hallucinogens might be formed after methylation of indole derivatives (*1*). Methylated products such as the hallucinogen dimethyltryptamine (DMT) have been investigated as possible causative agents of schizophrenia (*2*). Several investigators have reported the enzymatic synthesis of DMT by human brain, lung, and blood (*3*). In these studies *S*-adenosylmethionine, a major methyl donor in methylations of biogenic amines, was used (*4, 5*). There have been recent reports, starting with those of Laduron, that 5-methyltetrahydrofolic acid (5-MTHF) might also serve as a methyl donor for reactions of 5-hydroxytryptamine (serotonin) and tryptamine with a previously undescribed enzyme (*6, 7*). Indirect evidence suggested *O*- or *N*-methylation; however, the identity of the products was not established in all cases. We describe here the formation in vitro of tryptolines, a class of tricyclic compounds. They are the major products from the incubation of tryptamines and 5-MTHF with an enzymatic preparation from human brain. The products were identified by thin-layer chromatography, cocrystallization, gas-liquid chromatography, and mass spectrometry.

The enzymatic preparation from brain was prepared as follows. Autopsied human brain, primarily cortex, was homogenized at 4°C in five volumes of water that was distilled in quartz vessels. The homogenate was then centrifuged at 30,000*g* for 30 minutes. The supernatant was subjected to fractionation with ammonium sulfate; the material precipitating in 30 percent to 60 percent ammonium sulfate was taken up in 0.02*M* potassium phosphate buffer (*p*H 6.5) and dialyzed overnight against the same buffer. Incubation mixtures for the enzymatic assay contained, at final concentrations: 0.2*M* potassium phosphate buffer (*p*H 6.5); 0.015*M* amine substrate; 6 m*M* pargyline; 11 μM [^{14}C]5-MTHF (Amersham/Searle, 60 mc/mmole in 0.01*M* mercaptoethanol); and tissue extract, 1 to 2 mg protein. Human brain enzyme, heated to 95°C for 5 minutes, was used as a blank. The mixtures (final volume, 0.45 ml) were incubated for 60 minutes at 37°C. The reaction was terminated with 0.5 ml of 0.5*M* borate buffer (*p*H 10). Then 5 ml of toluene and isoamyl alcohol (97 : 3) was added, the mixture was shaken vigorously for 15 seconds, 3 ml of the organic phase was transferred to a counting vial, and the solvent was removed by evaporation (*5, 8*) at 40°C in a vacuum oven. The residue was dissolved in 2 ml of ethanol, and its radioactivity was determined by liquid scintillation counting in 10 ml of toluene phosphor. Each substrate yielded a single major radioactive product, as determined by thin-layer chromatography.

Table 1. Relative substrate specificity of a partially purified, enzymatic preparation of human brain with tryptamine activity at 100 percent (59 pmole per milligram of protein per hour). Products were examined only for tryptamine, *N*-methyltryptamine, and 5-hydroxytryptamine. Incubation products were extracted into 5 ml of toluene and isoamyl alcohol (97 : 3), except for 5-hydroxytryptamine, for which 5 ml of ethyl acetate was used.

Substrate	Relative activity
Tryptamine	100
N-Methyltryptamine	61
N,N-Dimethyltryptamine	3
5-Hydroxytryptamine	99
5-Methoxytryptamine	78
1-Methyltryptamine	100
Tryptoline	113
1-Methyltryptoline	5

Under these conditions enzymatic activity is linear with time for at least 2 hours and with protein up to 9 mg/ml, when a boiled tissue blank is used. The ratio of the reaction product to the boiled enzyme blank is at least 5 : 1. The optimal *p*H for the reaction is 6.5, and phosphate buffers of increasing strength enhance the reaction. As has been reported (*7*) methylcobalamin (0.025*M*) and flavin adenine dinucleotide (0.01*M*) also increased formation of the product. At 1 m*M* concentrations, ascorbic acid enhances the reaction by 30 percent, while 1 m*M* cupric ion inhibits it by 77 percent. The relative activities of several substrates (Table 1) demonstrate that tryptamine and 5-hydroxytryptamine are the most active substrates among the indolealkylamines tested. Methylation of the indole nitrogen does not affect the reaction rate; in contrast, methylation of the side chain nitrogen decreases activity. The apparent Michaelis-Menton constant (K_m) for the enzyme from brain was 4 m*M* for tryptamine; 6 m*M* for *N*-methyltryptamine; 2 m*M* for 5-hydroxytryptamine; and 3 m*M* for 5-MTHF when tryptamine was used as a substrate. A comparison of enzymatic activity in a supernatant preparation of several regions of human brain was made with tryptamine as substrate. The relative activity, in units per gram of tissue, was: hypothalamus, 100; medial thalamus, 0; septal region, 20; amygdala, 64; occipital cortex, 14; temporal cortex, 62. Enzymatic activity was also present in human platelets (*9*) and liver, but not red cells or plasma.

We have been particularly concerned with identifying the reaction products formed from tryptamine, *N*-methyltryptamine, and 5-hydroxytryptamine. The reaction has generally been thought to be a methyl transfer in which tryptamine would yield *N*-meth-

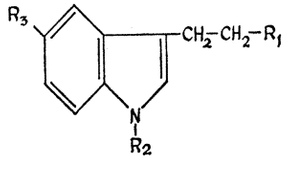
yltryptamine and DMT while the product from *N*-methyltryptamine would be DMT. We initially attempted to identify the products by thin-layer chromatography in an isopropanol, ammonium hydroxide, water system. Unexpectedly, the major product from tryptamine had an R_F of 0.30, while *N*-methyltryptamine and DMT had R_F values of 0.16 and 0.42, respectively. We therefore synthesized a variety of *N*-methylated derivatives, several of which have not been well characterized before (*10*). The R_F values (Fig. 1) suggest that none of these *N*-methylated derivatives is the product from either tryptamine or *N*-methyltryptamine. Similarly, we concluded that the product from 5-hydroxytryptamine was not an *N*- or *O*-methylated derivative.

We then turned our attention to the class of tricyclic compounds which we have named the tryptolines. This class has also been called: 1,2,3,4-tetrahydro-β-carbolines; 9*H*-1,2,3,4-tetrahydropyrido[3,4-b]indoles; and 1,2,3,4-tetrahydronorharmans. They occur naturally, but have not previously been reported to occur in human systems (*11*). Tryptoline, 1-methyltryptoline, and 5-hydroxytryptoline were synthesized (*12*). Tryptoline and the enzymatic product from tryptamine behaved identically in the six thin-layer chromatography solvent systems used. Similarly, 1-methyltryptoline and the product from *N*-methyltryptamine migrated identically in the three systems studied (see legend to Fig. 1). Five-hydroxytryptamine yielded a product which moved with 5-hydroxytryptoline in four systems. Furthermore, the radioactive products, isolated by thin-layer chromatography, were successively cocrystallized with the appropriate, authentic compound with a constant specific activity in three different solvent systems (*13*).

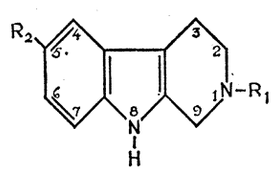
For final identification of two of these products, we used computer-controlled, gas-liquid chromatography combined with mass spectrometry to compare authentic tryptolines to nonradioactive, enzymatic products. A Finnigan 3200 quadrupole mass spectrometer and 6000 data system (Finnigan Corporation, Sunnyvale, California) were used (*14*). The enzymatic product from *N*-methyltryptamine gave a retention time and mass spectrum identical to that of 1-methyltryptoline (*15*).

To facilitate gas liquid chromatography, 5-hydroxytryptoline was first converted to a derivative with trifluoroacetic anhydride. The bis-trifluoroacetyl derivative compound had a retention time (*16*) and mass spectrum identical to the same derivative of the enzymatic product from 5-hydroxytryptamine (Fig. 2). For both, the molecular ion and base peak are at 380 amu. Other characteristic fragment ions are: 311 amu, from the loss of a CF_3 group; 283 amu, from the loss of CF_3CO; and 255 amu, from the loss of CF_3-$CONCH_2$. The fragment ion at 158 amu arises from the loss of an additional CF_3CO group from the 255 fragment ion.

We have, therefore, demonstrated the in vitro condensation of a one-carbon unit with the side-chain nitrogen of tryptamine, *N*-methyltryptamine, and 5-hydroxytryptamine, followed by cyclization to form a tryptoline. The sequence of the in vitro transfer reaction in mammalian tissues is unknown; it could consist of either entirely enzymatic, or some combination of enzymatic and nonenzymatic steps. For example, the sequence might be initiated with the formation of methanol, followed by an enzymatic oxidation to formaldehyde and a nonenzymatic condensation. Such a mecha-



	R_1	R_2	R_3	R_f
Tryptamine	$-NH_2$	H	H	0.24
N-Methyltryptamine	$-NHCH_3$	H	H	0.16
N,N-Dimethyltryptamine	$-N(CH_3)_2$	H	H	0.36
N,N,N-Trimethyltryptamine	$-\overset{\oplus}{N}(CH_3)_3$	H	H	0.003
1-Methyltryptamine	$-NH_2$	$-CH_3$	H	0.28
1-Methyl-N-methyltryptamine	$-NHCH_3$	$-CH_3$	H	0.18
1-Methyl-N,N-dimethyltryptamine	$-N(CH_3)_2$	$-CH_3$	H	0.45
1-Methyl-N,N,N-trimethyltryptamine	$-\overset{\oplus}{N}(CH_3)_3$	$-CH_3$	H	0.002
N-Methyl-5-Hydroxytryptamine	$-NH(CH_3)$	H	OH	0.14
5-Methoxytryptamine	$-NH_2$	H	$-O-CH_3$	0.24
5-Methoxydimethyltryptamine	$-N(CH_3)_2$	H	$-O-CH_3$	0.35



	R_1	R_2	R_f
Tryptoline	H	H	0.33
1-methyltryptoline	$-CH_3$	H	0.62
5-hydroxytryptoline	H	$-OH$	0.29
5-methoxytryptoline	H	$-O-CH_3$	0.32

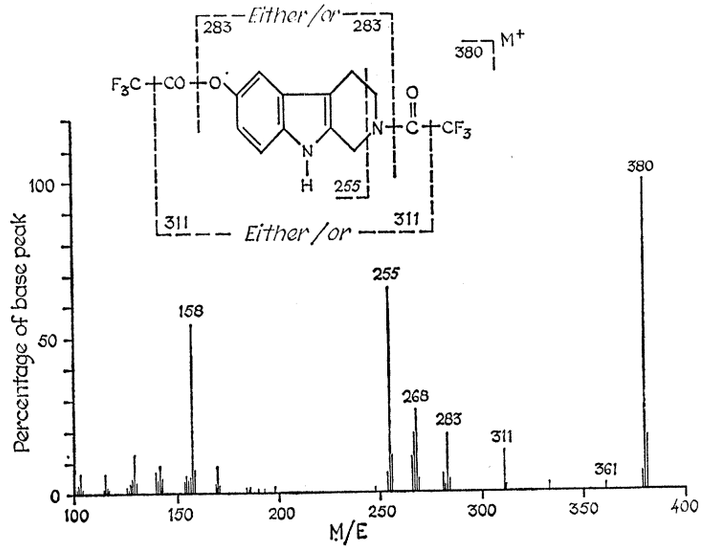


Fig. 1 (left). Chemical structures for selected tryptamine and tryptoline derivatives with R_F values on silica gel 254 (Brinkman Instruments, Westbury, New York) in methanol, (1*N*) ammonium hydroxide and water (100 : 20 : 10) (*21*). Fig. 2 (right). Mass spectrum of enzymatic product from 5-hydroxytryptamine. The spectrum is identical to that of authentic 5-hydroxytryptoline.

nism was recently proposed for *S*-adenosylmethionine and 5-hydroxytryptamine, where several products were suggested but none was identified (*17*). Or, an enzymatic oxidation of 5-MTHF to 5-formyl-THF might precede the release of free formaldehyde, followed by nonenzymatic condensation (*18*). A third sequence might involve the transfer of a formyl group directly from the enzyme, or coenzyme, to the substrate, with subsequent cyclization. Further experiments are required to elucidate the mechanism of the overall reaction sequence, to examine pharmacological actions of the products, and to determine whether they are formed endogenously.

Note added in proof: While in press similar enzymatic products have been described from tryptamines (*19*) and catecholamines (*20*).

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13. Successive cocrystallizations of the product from tryptamine with tryptoline from ethyl acetate, toluene, and acetone gave specific activities of 11,177, 10,966, and 10,370 disintegrations per minute per milligram. Cocrystallization of the product from *N*-methyltryptamine with authentic 1-methyltryptoline from the same solvents gave specific activities of 3468; 3358; and 3363 dpm/mg. Cocrystallization of the product from 5-hydroxytryptamine with authentic 5-hydroxytryptoline from methanol, a mixture of methanol and water (4 : 1), and 95 percent ethanol, yielded specific activities of 10,338, 8,809, and 10,533 dpm/mg.
14. We thank R. Skinner, J. Cornelius, J. Knight, and R. Finnigan for their help with the mass spectrometry.
15. The gas-liquid chromatography was performed on a U-shaped column (1.5 m by 2 mm) with 2 percent OV 17 on 80 to 100 mesh Gas Chrom 2; helium was the carrier gas, with a flow of 22 ml/min. The temperatures were 185°C for the column, and 250°C at the inlet and interface.
16. The gas-liquid chromatography was performed as in (*15*), except that the U-shaped column contained 3 percent OV 17 and the column temperature was 220°C.
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21. R_F values in other solvent systems examined: (i) In isopropanol, ammonium hydroxide (10 percent), and water (200 : 10 : 20), the R_F of tryptamine was 0.26; of *N*-methyltryptamine, 0.16; of DMT, 0.42; of tryptoline, 0.30; and of 1-methyltryptoline, 0.49. (ii) In *n*-Butanol, acetic acid and water (12 : 3 : 5), the R_F of tryptamine was 0.66; of *N*-methyltryptamine, 0.59; of DMT, 0.53; of tryptoline, 0.63; and of 5-methoxydimethyltryptamine, 0.45. (iii) In toluene, acetic acid, ethyl acetate, and water (80 : 40 : 20 : 5), the R_F of tryptamine was 0.18; of *N*-methyltryptamine, 0.17; of DMT, 0.10; and of tryptoline, 0.20. (iv) In methanol, tetrahydrofuran, and formic acid (50 : 50 : 1), the R_F of tryptamine was 0.52; of *N*-methyltryptamine, 0.42; of DMT, 0.30; of tryptoline, 0.44; and of 1-methyltryptoline, 0.21. (v) In acetone and ammonium hydroxide (99 : 1), the R_F of tryptamine was 0.47; of *N*-methyltryptamine, 0.13; of DMT, 0.18; of tryptoline, 0.14; and of 1-methyltryptoline, 0.44.
22. We thank S. O'Connor, P. Angwin, and E. Blanz, Jr., for assistance. Supported by NIH program-project grant MH 23861, NIH biosciences training grant fellowship MH 8304 (G.R.E.), and a research scientist development award MH 24161 (J.D.B.). R.J.W. has been a visiting faculty member, Department of Psychiatry, Stanford University. Send reprint requests to R. J. Wyatt, Laboratory of Clinical Psychopharmacology, National Institute of Mental Health, WAW Building, St. Elizabeths Hospital, Washington, D.C. 20032.

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Primate Type C Virus p30 Antigen in Cells from Humans with Acute Leukemia

Abstract. *Antigens related to the major structural protein (p30) of type C viruses isolated from a woolly monkey and a gibbon ape were found in peripheral white blood cells from five patients with acute leukemia.*

Several lines of evidence have suggested that certain forms of acute leukemia (AL) in man may be associated with type C viruses. Cells from patients with acute leukemia contain an enzyme with biochemical properties related to those of known type C viruses and with antigenic properties similar to those of the polymerases of the woolly monkey type C virus (SSAV) and the gibbon ape leukemia virus (GALV) (*1*). The DNA products of endogenous reactions from particulate fractions of AL cells hybridize preferentially to viral RNA from SSAV and GALV (*2*). While the woolly monkey type C virus represents a single isolate from a pet with fibrosarcoma, there are several independent isolates of type C viruses from gibbons (*3*). The woolly monkey sarcoma virus produces sarcomas and brain tumors in marmosets, and GALV has produced acute myelogenous leukemia in a normal gibbon (*4*).

Radioimmunoassays can detect antigens related to the major structural proteins (p30) of type C viruses in tissues of primates (*5*) and man (*6*, *7*). The data presented here show that cells from patients with AL contain p30 antigens specifically related to viruses of the SSAV-GALV group. Peripheral blood leukocytes (provided by R. Gallo and R. Gallagher) which had been obtained from patients by leukophoresis were stored at −20°C prior to use. The cells (1 to 5 g, wet weight, packed) were thawed, extracted, and partially purified by gel filtration (*5*, *6*). These extracts were used as competing antigens in radio-