AMINO ACID AND PEPTIDE DERIVATIVES OF THE INDOLE SERIES

III. Synthesis and Properties of Analogs of α-Methyltryptamine

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Khimiya Prirodnykh Soedinenii, Vol. 3, No. 2, pp. 108–116, 1967

DL-α-Methyltryptamine hydrochloride, which is known in Soviet medical practice under the name Indopan is an active stimulator of the central nervous system [1, 2].

Indopan is characterized by interesting physiological and pharmacological properties. It enhances the motive activity of animals, shortens the time of action of narcotics, weakens the sedative and hypothermal action of reserpine, possesses a high antimonoamine oxidase activity, and also gives satisfactory results when used as an antidepressant in the treatment of a number of psychiatric diseases [3–6]. These facts have stimulated the development of our work on the synthesis and detailed study of a number of its amino acid and peptide derivatives.

Table 1

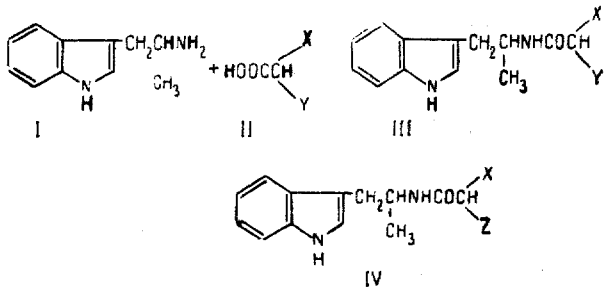
Compounds of Type (II)

Compound no.	X	Y	Literature data	Compound no.	X	Y	Literature data
(IIa)	NHCBz*	H	[9]	(IIe)	NHCBz	$(CH_2)_2CO_2Bz$	[12]
(IIb)	CH_2NHCBz	H	[10]	(IIf)	NHCBz	$(CH_2)_2CONH_2$	[13]
(IIc)	$(CH_2)_2NHCBz$	H	[11]	(IIg)	NHCBz	$(CH_2)_2CO_2Me$	[12]
(IId)	$CH(OH)CH_2NPI$	H	—	(IIh)	CH_2CHCO_2Bz | NHCBz	H	[14]

* Here and below, CBz represents carbobenzoxy, Pl phthalyl, Bz benzyl, and Me methyl.

We have previously [7, 8] investigated the amino acid and peptide derivatives of 5-methoxytryptamine and have shown that the introduction of an amino acid or peptide residue into the side-chain of this biogenic amine leads to materials with less pronounced pharmacological properties. Consequently, it appeared desirable to us to synthesize a number of amino acid and peptide derivatives of α-methyltryptamine (I).

The present paper describes a method for obtaining amino acid and peptide derivatives of type (IV), and describes the results of pharmacological and biochemical studies of them. Synthesis was effected by the route shown below:



The symbols X, Y, and Z for compounds (II), (III), and (IV) are given in Tables 1, 2, and 3.

In the synthesis of amino acid derivatives of type (III) (which may also be considered as amide derivatives of amino acids), we studied the methods generally used for joining a peptide chain: the dicyclohexylcarbodiimide method, the acid chloride method, and the mixed anhydride method. The last proved to be the most convenient, giving the highest yields and the purest products. Consequently, most compounds of type (III) (see Table 2) were prepared by the

condensation of α-methyltryptamine in ethyl acetate solution with protected amino acids of type (II). The subsequent elimination of the protecting groups was carried out, in the case of the carbobenzoxy group, by hydrogenation with hydrogen in the presence of 10% Pd/C. Where the phthalyl group was used, it was split off by hydrazinolysis.

Table 2

Compound of Type (III)

Compound no.	X	Y	Compound no.	X	Y
(IIIa)	NHCBz	H	(IIIf)	NHCBz	$(CH_2)_2CONH_2$
(IIIb)	CH_2NHCBz	H	(IIIg)	NHCBz	$(CH_2)_2CO_2Me$
(IIIc)	$(CH_2)_2NHCBz$	H	(IIIh)	CH_2CHCO_2Bz NHCBz	H
(IIId)	$CH(OH)CH_2NPl$	H			
(IIIe)	NHCBz	$(CH_2)_2CO_2Bz$	(IIIi)	$NHCOCHNHCBz$ $(CH_2)_2CO_2Me$	$(CH_2)_2CO_2Bz$
			(IIIj)	$NHCOCHNHCBz$ $(CH_2)_2CO_2Me$	$(CH_2)_2CO_2Me$

L-α-Glutamyl-L-α-glutamyl-α-methyltryptamine (IVi) was synthesized by condensing the γ-methyl ester of L-α-glutamyl-α-methyltryptamine (IVg) with the γ-benzyl (IIe) or γ-methyl (IIg) ester of CBz-L-glutamic acid with subsequent saponification of the ester groups with alcoholic potassium hydroxide and catalytic hydrogenation of the CBz-L-α-glutamyl-L-α-glutamyl-α-methyltryptamine.

The hydrazide of L-α-glutamyl-α-methyltryptamine (IVj) was obtained by the action of hydrazine hydrate on the γ-methyl ester of L-α-glutamyl-α-methyltryptamine (IVg).

Table 3

Compounds of Type (IV)

Compound no.	X	Z	Compound no.	X	Z
(IVa)	$NH_2 \cdot CH_3COOH$	H	(IVg)	$NH_2 \cdot CH_3COOH$	$(CH_2)_2CO_2Me$
(IVb)	$CH_2NH_2 \cdot C_4H_6O_6 \cdot H_2O$	H	(IVh)	CH_2CHCO_2H $NH_2 1/2H_2O$	H
(IVc)	$(CH_2)NH_2 \cdot C_4H_6O_6 \cdot H_2O$	H			
(IVd)	$CH(OH)CH_2NH_2 \cdot C_4H_6O_6 \cdot H_2O$	H	(IVi)	$NHCOCHNH_2 \cdot 1/2H_2O$ $(CH_2)_2CO_2H$	$(CH_2)_2CO_2H$
(IVe)	$NH_2 \cdot 1/2H_2O$	$(CH_2)_2CO_2H$			
(IVf)	$NH_2 \cdot C_4H_6O_6$	$(CH_2)_2CONH_2$	(IVj)	$NH_2 \cdot C_4H_6O_6$	$CH_2CONHNH_2 \cdot C_4H_6O_6$

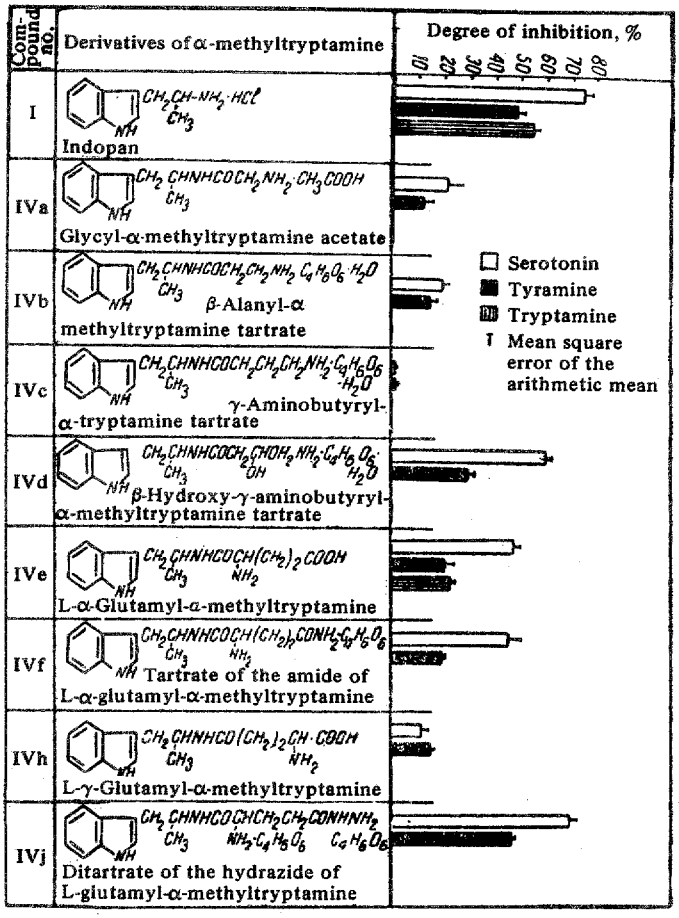
In order to obtain water-soluble crystalline materials more suitable for biological investigations, the bases (IVa)-(IVd), (IVf), (IVg), and (IVj), formed on hydrogenation were converted into salts of one kind or another (Table 3).

The pharmacological investigations of the compounds (IVa)-(IVh) synthesized were carried out with respect to a number of properties characteristic for Indopan. We have studied the influence of substances of type (IV) on the general state of the animals, the arterial pressure, the lumen of the vessels, and the tone of the third eyelid. The interaction of these compounds with narcotics (hexenal) and stimulants (amphetamine) and also their influence on the action of tryptamine have been demonstrated. The toxicity of these compounds has been studied and their antagonism to reserpine has been elucidated.

As a result, it has been found that the amino acid and peptide derivatives of Indopan possess a marked pharmacological activity and with respect to the nature of their action and activity those closest to Indopan are glycyl-α-methyltryptamine (IVa) and L-α-glutamyl-α-methyltryptamine (IVe). Like Indopan, they possess a stimulating action on the central nervous system, i.e., they raise the motive activity, the reflector excitability, and hyperthermia, shorten the action of narcotics, and have the phenomenon of "group toxicity."

Compounds (IVa)-(IVh) also possess the elements of peripheral sympathominimetic action: they raise the arterial

pressure, cause a contraction of the third eyelid, and constrict the peripheral vessels. With a less pronounced influence on the peripheral adrenoreactive system of the organism, substances (IVa) and (IVe) exert a milder and more prolonged action on the central nervous system than does Indopan. As a reserpine antagonist, compound (IVe) is scarcely inferior to Indopan, and it is even somewhat superior to it in its capacity for enhancing the tremor caused by tryptamine. L-α-



Influence of amino acid derivatives of α-methyltryptamine in a concentration of 1×10^{-3} M on the activity of the mytochondrial monoamine oxidase of rat liver.

Glutamyl-α-methyltryptamine (IVe) has a low toxicity in comparison whth that of Indopan. Thus, when this compound is adminstered internally to white mice, its LD_{50} is 57 mg/kg (the LD_{50} for Indopan [1] is 38 mg/kg).

In contrast to Indopan and L-α-glutamyl-α-methyltryptamine, no exciting action on the central nervous system is exerted by β-alanyl-α-methyltryptamine (IVb), γ-aminobutyryl-α-methyltryptamine (IVc), and β-hydroxy-γ-aminobutyryl-α-methyltryptamine (IVd); however, on the other hand, they possess a weak tranquilizing action. In addition, these compounds enhance the hyperthermal and exciting action of amphetamine and increase its toxicity.

It is interesting that compounds (IVb)-(IVd) also cause a weakening of the skeletal musculature in white mice, rats, and cats, which is connected with a disturbance of the transfer of nervous excitation from the nerve termination to the muscle.

In addition to the pharmacological study of the amino acid derivatives of α-methyltryptamine, we have also carried out chemical investigations of compounds (IVa)-(IVh) and (IVj) (figure.) It has been shown that seven of the eight derivatives of α-methyltryptamine inhibit the activity of monoamine oxidase in vitro. The most active in this respect was the hydrazide of L-α-glutamyl-α-methyltryptamine (IVj) which is hardly inferior to Indopan. In a concentration of 1×10^{-3} M, γ-aminobutyryl-α-methyltryptamine (IVc) causes no inhibition of the deamination of serotonin and tyramine by the mitochondria of rat liver. The remaining six derivatives of α-methyltryptamine, (IVa), (IVb), (IVd)-(IVf), and (IVh), inhibit the deamination of serotonin to a greater extent than the deamination of tyramine and tryptamine.

The yields, melting points, and other characteristics of the compounds obtained are given in Tables 4 and 5.

Table 4

Physicochemical Characteristics of the Compounds of Types (II) and (III)

Compound no.	Name	Formula	Yield, %	Mp, °C	Solvent for recrystallization	$[\alpha]_D^{20}$, deg	R_f ref. to (IIIa)	
							EA**	EA–acetone
(IId)	N-Pl-β-hydroxy-γ-aminobutyric acid	$C_{12}H_{11}O_5N$	60	128—129	Benzene–EA–petroleum ether (4:1:5)	—	—	—
(IIIa)	CBz-glycol-α-methyltryptamine	$C_{21}H_{23}O_3N_3$	75	119—121	Water–acetone	—	1.0	1
(IIIb)	CBz-β-alanyl-α-methyltryptamine	$C_{23}H_{25}O_3N_3$	80	139—140	Benzene	—	1.5	1
(IIIc)	CBz-γ-aminobutyryl-α-methyltryptamine	$C_{23}H_{27}O_3N_3$	60	92—94	Water–alcohol	—	1.5	1
(IIId)	Pl-β-hydroxy-γ-aminobutyryl-α-methyltryptamine	$C_{23}H_{23}O_4N_3$	54	Amorph.		—	2.2	1.3—1.4
(IIIe)	γ-benzyl ester of CBz-L-α-glutamyl-α-methyltryptamine	$C_{31}H_{33}O_5N_3$	87	124—126	Benzene–hexane (1:1)	+9.8 (*c* 1; THF)	1.9	1.2—1.3
(IIIf)	CBz-L-glutamyl-α-methyltryptamine	$C_{24}H_{28}O_4N_4$	70	126—127	Water–alcohol	—7.5 [*c* 1; CH_3OH—THF (1:1)]	3.5	1.6
(IIIg)	γ-methyl ester of CBz-L-α-glutamyl-α-tryptamine*	$C_{25}H_{29}O_5N_3$	70	128—120		+9.2 (*c* 1.5; THF)	1.8	1.2—2.3
(IIIh)	α-benzyl ester of the CBz-L-glutamyl-γ'-benzyl ester of L-α-glutamyl-α-methyltryptamine	$C_{31}H_{33}O_5N_3$	60	82—84	EA–petroleum ether (1:1)	—14.5 (*c* 1.2; CH_3OH)	1.8—1.9	1.3
(IIIi)	γ-methyl ester of the CBz-L-glutamyl-γ'-benzyl ester of L-α-glutamyl-α-methyltryptamine	$C_{37}H_{42}O_8N_4$	50	178—180	Water–DMF	—7.7 (*c* 1; DMF)	—	1.1
(IIIj)	γ,γ'-dimethyl ester of CBz-L-α-glutamyl-L-α-glutamyl-α-methyltryptamine	$C_{31}H_{33}O_8N_4$	52	104—106	Benzene–ether (1:1)	—9.5 (*c* 1; CH_3OH)	—	1.1

* The condensation was effected by the dicyclocarbodiimide method; the yield when condensation was carried out by the mixed anhydride method was 51%.

** EA – ethyl acetate; THF – tetrahydrofuran; DMF – dimethylformamide.

Table 5

Physicochemical Characteristics of Compounds of Type (IV)

Compound no.	Name	Formula	Yield, %	Mp, °C	Solvent for recrystallization	$[\alpha]_D^{20}$, deg	R_f in systems	
							1	2
(IVa)	Glycyl-α-methyltryptamine acetate	$C_{19}H_{21}O_3N_3$	84	151—152	Abs. alcohol	—	0.59	0.64
(IVb)	β-Alanyl-α-methyltryptamine tartrate	$C_{18}H_{27}O_8N_3$	75	98—99	Abs. alcohol–abs. ether (1:4)	—	0.61	0.42
(IVc)	γ-Aminobutyryl-α-methyltryptamine tartrate	$C_{19}H_{29}O_8N_3$	64	110—114	Abs. alcohol–abs. ether (1:4)	—	0.66	0.43
(IVd)	β-Hydroxy-γ-aminobutyro-α-methyltryptamine tartrate	$C_{19}H_{29}O_9N_3$	80	143—146	Abs. alcohol–abs. ether (1:4)	—	0.59	0.40
(IVe)	L-α-Glutamyl-α-methyltryptamine	$C_{16}H_{21}O_3N_3$	85	125—126	Abs. alcohol–EA (1:1)	+20.6 (*c* 1.45; CH_3OH)	0.67	0.49
(IVf)	L-α-Glutamyl-α-methyltryptamine tartrate	$C_{20}H_{28}O_8N_4$	52	75—77	Abs. alcohol–abs. ether (1:4)	+26 (*c* 1; CH_3OH)	0.56	0.55
(IVh)	L-γ-Glutamyl-α-methyltryptamine	$C_{16}H_{21}O_3N_3$	75	181—183	Abs. alcohol–abs. ether (1:2)	+8.3 (*c* 1; 50% CH_3OH)	0.64	0.48
(IVi)	L-α-glutamyl-L-α-glutamyl-α-methyltryptamine	$C_{21}H_{30}O_7N_4$	50	104—106	Abs. alcohol–abs. ether (1:3)	+19.5 (*c* 1.1; CH_3OH)	0.17	—
(IVj)	Ditartrate of the hydrazide of L-α-glutamyl-α-methyltryptamine	$C_{24}H_{35}O_{18}N_5$	25	149—151	Abs. alcohol–abs. ether (1:2)	+26 (*c* 1; CH_3OH)	0.55	0.67

Experimental

The R_f values of the protected derivatives were determined on neutral alumina (activity grade II) in ethyl acetate or ethyl acetate – acetone (1:1) relative to the R_f value of CBz-glycyl-α-methyltryptamine, and the R_f values for the free bases on Leningrad type M paper in the following systems: 1) $C_4H_9OH - CH_3CHOOH - H_2O$ (4:1:5) and 2) C_5H_5N – iso – $C_5H_{11}OH - H_2O$ (10:10:7). The results of elemental analysis of all the compounds synthesized agreed with the calculated figures.

N-Phthalyl-β-hydroxy-γ-aminobutyric acid (IId). Five grams of β-hydroxy-γ-aminobutyric acid [15] was fused with 7 g of phthalic anhydride at 168°–170° C for 1 hr. The resulting brown mass was dissolved in acetone, the solution was filtered, the solvent was distilled off in vacuum to dryness, the residue was dissolved in 50–70 ml of ethyl acetate, and compound (IId) was precipitated with petroleum ether.

Compounds of type (III). To an ethyl acetate solution of 0.01 mole of a protected amino acid of type (II) cooled to −10° C was added 0.014 mole of triethylamine and then, after 5 min, 0.014 mole of isobutyl chlorocarbonate. The reaction solution was stirred at −10° C for 10–15 min and then 0.01 mole of α-methyltryptamine (I) dissolved in 50–70 ml of ethyl acetate was added to it. Stirring was continued for 30–40 min at −10° C and for 2–3 hr at 20° C, after which the reaction solution was left at room temperature until the following day. The precipitate of triethylamine hydrochloride that had deposited was filtered off and the filtrate was washed successively with 0.5 N hydrochloric acid, 5% aqueous sodium hydrogen carbonate, and water; it was then dried over anhydrous sodium sulfate, the ethyl acetate was distilled off in vacuum, and the compound of type (III) was obtained by triturating the residue with hexane or ether.

Compounds of type (IV). In the presence of 10% Pd/C, 0.01 mole of a compound of type (III) was hydrogenated for 2–4 hr in 100 ml of methanol [with the addition of a few drops of glacial acetic acid in the case of compound (IIIg)] until the absorption of hydrogen ceased. The catalyst was filtered off, the solvent was distilled off in vacuum, the residue was dissolved in 20–30 ml of absolute ethyl alcohol, the solution was treated with 0.014 mole of acetic or tartaric acid, and the resulting salt was isolated by the addition of absolute ether or ethyl acetate.

β-Hydroxy-γ-aminobutyryl-α-methyltryptamine tartrate (IVd). A solution of 0.01 mole of compound (IIId) in 50 ml of absolute ethyl alcohol was boiled with 1 ml of hydrazine hydrate for 1 hr. The precipitate of phthalyl hydrazide that deposited was filtered off, the solvent was distilled off in vacuum, the oily residue was dissolved in ethyl acetate, the solution was filtered, the filtrate was evaporated in vacuum, the residue was treated with 20 ml of absolute ethyl alcohol containing 0.015 mole of tartaric acid, and the alcoholic solution was poured into 100 ml of absolute ether cooled to 0° C. The tartrate (IVd) that precipitated was filtered off and reprecipitated from alcohol with ether.

γ,γ'-Dimethyl ester of CBz-L-glutamyl-L-glutamyl-α-methyltryptamine (IIIj). A solution of 1.2 g of compound (IIg) in 40 ml of tetrahydrofuran was cooled to −10° C and then 0.7 ml of triethylamine and, after 5 min, 0.75 ml of isobutyl chlorocarbonate, were added. The reaction solution was stirred at −10° C for 10 min and then 1.5 g of compound (IVg) in 10 ml of tetrahydrofuran and another 0.7 ml of triethylamine were added. The subsequent procedure was as described for the compounds of type (III).

The γ-benzyl ester of the CBz-glutamyl-γ'-methyl ester of L-glutamyl-α-methyltryptamine (IIIi) was obtained similarly. In this case, the starting compounds were (IIe) and (IVg).

L-α-Glutamyl-L-α-glutamyl-α-methyltryptamine (IVi). A solution of 3 g of compound (IIIi) in 8 ml of 10% alcoholic caustic potash was left at 20° C for 30 min and was then acidified to pH 5–6 with 1 N hydrochloric or acetic acid. The oil hat deposited was extracted with ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The solvent was distilled off in vacuum and the residue was dissolved in 50 ml of methyl alcohol and hydrogenated over 10% Pd/C until the absorption of hydrogen ceased. The catalyst was filtered off, the solution was poured into absolute ether cooled to 0° C, and compound (IVi) was filtered off and purified by reprecipitation from ethanol with ether.

Hydrazide of L-glutamyl-α-methyltryptamine (IVj). A solution in 10 ml of methyl alcohol of 4.7 g of the γ-methyl ester of L-glutamyl-α-methyltryptamine (IVg), obtained by the hydrogenation of 7 g of the γ-methyl ester of CBz-L-glutamyl-α-methyltryptamine (IIIg), was treated with 1.5 ml of hydrazine hydrate. The resulting solution was boiled for 1 hr and cooled, left at 20° C for 10–12 hr, and filtered. 4 g of tartaric acid dissolved in 20 ml of methyl alcohol to the filtrate was added, the alcoholic solution was poured into 100 ml of absolute ether, and the compound (IVj) which separated out was filtered and purified by reprecipitation from ethanol with ether.

Deamination of the amino acid derivatives of α-methyltryptamine with monoamine oxidase. To a suspension of 6 mg of the lyophilized mitochondrial membranes of rat liver [16] in 0.5 ml of 0.1 M phosphate buffer (pH 4) was added 0.18 ml of one of the inhibitors of type (IV) in a concentration of 1×10^{-2} M (the final concentration of the inhibitor in the sample was 1×10^{-3} M) one of the substrates, tyramine 3.2×10^{-2} M; serotonin, 5×10^{-2} M; or tryptamine, 3.0×10^{-1} M) [17] and 0.1 M (pH 7.4) phosphate buffer was added to the sample to make a volume of 1.8 ml.

The samples were incubated in an atmosphere of oxygen at 37° C in a Warburg apparatus for 45 min. The proteins were precipitated by the addition of 50% trichloroacetic acid solution to give a final concentration of 5%. The total volume of the sample was 2 ml. The protein precipitate was separated by centrifuging at 10 000 rpm for 2 min in a TsUM-1 centrifuge. In control samples (with one substrate and without an inhibitor) in which the deamination reaction took place by a first-order equation during 45 min of incubation, the following amounts of ammonia were liberated: 2.5 μmole for tyramine, 1.4 μmole for serotonin, and 1.6 μmole for tryptamine.

The arithmetic mean values obtained in 3 or 4 parallel experiments characterized the degree of inhibition of the above monoamines (in percentages with respect to the control samples without inhibitors) (figure).

Summary

1. A method for obtaining amino acid and peptide derivatives of α-methyltryptamine have been described.

2. In respect of their biological activity, the closest to Indopan are glycyl-α-methyltryptamine and L-α-glutamyl-α-methyltryptamine, which, at the same time, possess a weaker action on the peripheral adrenoreactive system.

3. When amino acid residues are introduced into the molecule of α-methyltryptamine, not only does the degree of biological activity of the compound change but, in some cases, new biological properties actually appear.

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2 June 1966

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