ESTABLISHMENT OF THE ABSOLUTE CONFIGURATION OF THE DIASTEREOISOMERS OF L'-α'-GLUTAMYL-α-METHYLTRYPTAMINE AND COMPARISON OF THEIR PHARMACOLOGICAL PROPERTIES WITH THE PROPERTIES OF INDOPAN

M. M. Vigdorchik, T. K. Trubitsyna,
N. V. Gavrilova, K. F. Turchin,
N. N. Suvorov, and M. D. Mashkovskii

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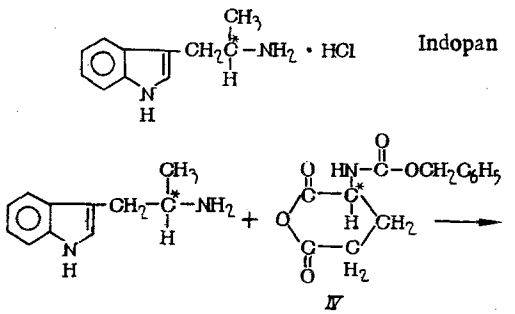
We have previously reported on the synthesis of a number of amino acid derivatives of DL-α-methyltryptamine [1], which is the basis of the psychotropic preparation, indopan [2]. Among these derivatives, the corresponding α-amide of L-glutamic acid [3], which has received the name "glutramine," has attracted the greatest interest from the pharmacological point of view.

This material was prepared by the action of DL-α-methyltryptamine (I) an a mixed anhydride of γ-benzyl N-carbobenzoxy-L-glutamate, with subsequent removal of the protective groups from the α-amide formed [1]. At this time, establishment of the configuration of glutramine was not carried out.

In the present work, with the objective of establishing the absolute configuration of glutramine and of synthesizing its diastereoisomer, we have studied the reaction of the inner anhydride of N-carbobenzoxy-L-glutamic acid (IV) [4] with DL-α-methyltryptamine (I) and with L-(–)-α-methyltryptamine (II). Separation of the enantiomers of α-methyltryptamine was effected by the method previously described [5].

It turned out that, as a result of the interaction of anhydride IV with the racemic modification I in chloroform in the presence of traces of acetic acid, a dextrorotatory crystalline compound V with a sharp melting point separated from the solution; after removal of the carbobenzoxy group by hydrogenolysis this leads to compound VII, which is absolutely identical to the glutramine preparation obtained by the method previously described [1]. Thereupon, after isolation of compound V, in addition to resinous materials a compound remains in the mother liquor which has the same electrophoretic and chromatographic mobility (in systems A and B; see experimental section) as V; we were not able to isolate it in the crystalline state or to purify it satisfactorily.



I, II, D-(+)-α-methyltryptamine (III)

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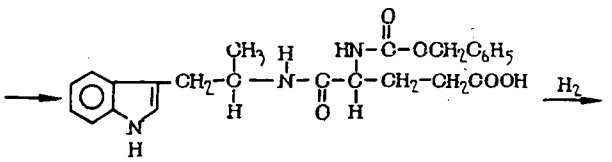
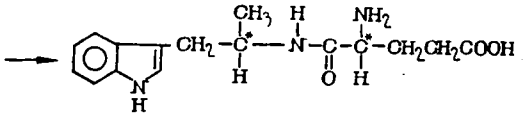
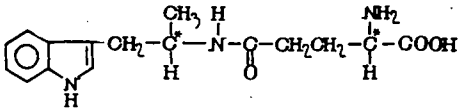


(+)-N'-carbobenzoxy-L'-α'-glutamyl-D-α-methyltryptamine (V)
(−)-N'-carbobenzoxyl-L'-α'-glutamyl-L-α-methyltryptamine (VI)



(+)-L'-α'-glutamyl-D-α-methyltryptamine (glutramine) (VII)
(−)-L'-α'-glutamyl-L-α-methyltryptamine (diastereoisomer of glutramine) (VIII).



L'-γ'-glutamyl-L-α-methyltryptamine (IX)

In distinction from racemate I, the reaction of anhydride IV with compound II under the same conditions leads to the formation of an amorphous levorotatory substance having a quite satisfactory elemental analysis for N-carbobenzoxyglutamic acid methyltryptamide (VI). As a result of removal of the protective group from this substance by hydrogenolysis we obtained an amorphous compound VIII which is, from NMR spectroscopic data (see below), an α-amide of glutamic acid, but differs from glutramine in being levorotatory and in the parameters of its NMR spectrum.

The absolute configuration* of the enantiomers of α-methyltryptamine has already been determined previously by use of ORD [5]. Therefore the absolute configurations of compounds VI and VIII are obvious: These are, respectively, N'-carbobenzoxy-L'-α'-glutamyl-L-α-methyltryptamine and L'-α'-glutamyl-L-α-methyltryptamine. Hence, however, it follows unavoidably that compound V is N'-carbobenzoxy-L'-α'-glutamyl-D-α-methyltryptamine, and glutramine is L'-α'-glutamyl-D-α-methyltryptamine. An additional proof of the indicated absolute configuration of glutramine is the fact that (+)-D-α-methyltryptamine (III) with anhydride IV forms a substance which does not differ from compound V in melting point or specific rotation.

As was to be expected, glutramine VII and its diastereoisomer VIII are easily distinguished by the use of NMR spectroscopy, the signals from the protons of the amino acid residue in the NMR spectrum of glutramine (VII) lying at lower field, and those from the α-methyl group and the α proton of the tryptamine fragment lying at higher field as compared with the positions of these same signals in the NMR spectrum of diasteroisomer VIII (see Table 1).

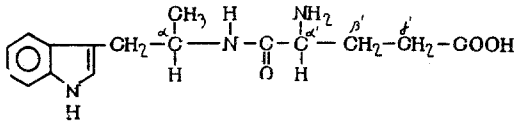
The difference in magnitude of the chemical shifts which was indicated above reaches 0.8 ppm for the protons of the β'-CH_2 group (in 2 N DCl), and this is probably caused by a different spatial orientation of the amino acid residue relative to the indole nucleus in glutramine and its diastereoisomer.

It should be noted that the results described above from the reaction of anhydrive IV with α-methyltryptamine indicate a preferential or even a prevailing formation of the α amides V and VI in this case.

Only in a single one of the experiments, in purifying diastereoisomer VIII did we isolate a small amount (about 0.02 g) of a crystalline substance having a mp of 150-152°C, to which one may assign the structure of a γ amide, i.e., L'-γ'-glutamyl-L-α-methyltryptamine (IX).

*Assignment to the D_G or L_G series, which in this case corresponds to the Kahn–Ingold–Prelog R or S configuration.

TABLE 1. Chemical Shifts of Protons in NMR Spectra of Glutramine (VII) and Its Diastereoisomer (VIII) (δ, ppm)



Compound	α'	β'	γ'	CH_3	α	β	Indole
VII	3,85	1,60	1,60	1,24	4,30	2,80	7,0—7,6
VIII	3,92	2,43	2,12	1,14	4,16	2,90	7,0—7,6
Δ	—0,07	—0,83	—0,52	+0,10	+0,014	—0,10	

Note. Solvent, 2 N DCL; internal standard, $(CH_3)_3COH$ (δ_{CH_3} = 1.23 ppm).

EXPERIMENTAL

Pharmacology

The pharmacological investigation of glutramine VII and its diastereoisomer VIII was carried out in comparison which indopan, which has been confirmed for use in medical practice as a stimulator of the central nervous system and an antidepressant [2, 3].

The investigation was conducted with respect to indices characteristic of action on the central and vegetative nervous systems. The experiments were conducted on mice, rats, rabbits, and cats, with injection of the substances intravenously, subcutaneously, intraperitoneally, and also into isolated organs.

It was ascertained that VII and VIII, like indopan, exert a stimulating action on the central nervous system: They increase motor activity and reflex excitability, and cause stereotypy, intensification of salivation, expansion of the pupils, and exophthalmia. Indopan is the most active in these respects; its initial action is observed when injected intravenously in a dose of 3-5 mg/kg; the action of VII is noted starting at a dose of 15 mg/kg and that of VIII, starting from 25 mg/kg. Stereotypy develops in mice at a dose of indopan of 5-10 mg/kg, of 20 mg/kg of VII, and of 30 mg/kg of VIII.

The lower stimulating activity of VIII as compared with indopan and VII is accompanied by a decrease in toxicity. The LD_{50} on injection intravenously to white mice, and calculated by the Kerber method, is 45 mg/kg for indopan, 82.5 mg/kg for VII, and 122.5 mg/kg for VIII.

The lower stimulating activity of VIII as compared with VII, and especially with indopan, is confirmed on study of the motor activity of white mice by the actometry method, which was measured over a 15-min period 1 h after intravenous injection of the preparations in equitoxic amounts (1/3 LD_{50}). The actometer counter readings in arbitrary units for the control group were 240 (126-354); on injection of indopan, 1825 (1677-1973); on injection of VII, 1108 (928-1288); and on injection of VIII, 796 (654-938).

In hyperthermal effect the glutamyl derivatives are also less active than indopan. On intravenous injection to white mice, indopan in a dose of 15 mg/kg causes a temperature elevation of 2°, but VII in a dose of 40 mg/kg causes a temperature elevation of 1°, with a duration of hyperthermy of about 3 h. Compound VIII did not exert a hyperthermal action in a dose of 40 mg/kg.

Preliminary (1 h) intraperitoneal injection of the preparations in equimolecular doses (20 mg/kg of indopan; 30 mg/kg of VII or VIII) retards the onset of ptosis and of the hypothermal action of reserpine (2 mg/kg, under the skin). In this characteristic indopan is the most active, and VIII is the least active. The magnitude of the ptosis in units by the method of Rubin et al. [6] 4 h after injection of indopan was 1.4, after injection of VII 2.4%, and after injection of VIII 2.6.

Like indopan and glutramine, its diastereoisomer VIII shortens the soporific action of hexenal (60 mg/kg, intravenously); however, it is inferior to them in this respect. While the duration of the soporific action of hexenal in the control group was 31 min, when indopan,

VII, or VIII had been preliminarily injected it was shortened to 8.5, 12.2, and 23.6 min, respectively.

An important feature of VII as compared with indopan is its less intense peripheral adrenomimetic action. Diastereoisomer VIII does not exert a stimulating action at all on the peripheral adreno-reactive systems of the organism: It does not cause elevation of arterial pressure, constriction of the peripheral vessels, or contractions of the third eyelid. An investigation of the antimonoaminooxidase activity in experiments *in vivo* by the Tedeschi method [7] shows that VII is essentially not inferior to indopan in activity (the ED_{50} for indopan on intravenous injection is 9.5 mg/kg; that of VII is 10.5 mg/kg), while the glutramine diastereoisomer VIII in a dose of 10-20 mg/kg does not change the convulsive action of tryptamine; that is, it does not exert an effect on its deamination. This fact makes it possible to suggest that, in distinction from glutramine, for steric reasons its diastereoisomer is not a substrate for monoaminooxidase. On isolated rat uterus horns indopan exerts a spasmogenic action starting at a concentration of 10^{-7} M; compound VII, starting at 10^{-6} M; and VIII, starting at 10^{-5} M. Thus, even in effect on the serotoninergic systems diastereoisomer VIII is inferior to VII.

Thus, in all the parameters studied the glutamyl-substituted indopans proved less active and less toxic than indopan. Thereupon diastereoisomer VIII proved significantly less active than VII. Compound VII, called glutramine, was transmitted to clinics for a study of its effectiveness in treating mentally ill patients. The clinical studies showed that glutramine has antidepressive activity, but is inferior to indopan. Diastereoisomer VIII is considerably less active; therefore, clinical tests of it were not carried out.

Chemistry

The NMR spectra were taken on a JNM-4H-100 spectrometer having a working frequency of 100 MHz. Values of $[\alpha]_D$ were determined with the aid of an A-1-EPL VNIIÉKI Prodmash automatic polarimeter. For thin-layer chromatography we used Silufol plates and the following solvent systems: A) isopropanol–benzene–aqueous ammonia (10:5:1); B) ethanol–aqueous ammonia (4:1). Electrophoresis on paper was conducted with the aid of an EMIB instrument at a field voltage of 7-6 V/cm, in a carbonate–bicarbonate buffer solution having pH 9.2. The travel of tryptophane was taken for E = 1.0. The developer in the thin-layer chromatography and in electrophoresis was the Ehrlich reagent.

(+)-N'-Carbobenzoxy-L'-α'-glutamyl-D-α-methyltryptamine (V). a) To a solution of 1.0 g of anhydride IV in 11 ml of chloroform was added a solution of 0.7 g of I in 5.5 ml of chloroform, and then a drop of acetic acid was added. The mixture was periodically stirred for 3 h at 20°; the precipitate formed was filtered off, washed with chloroform, and dried. Compound V was obtained (0.54 g, 31%)* having mp 183-185°; $[\alpha]_D^{20}$ + 6° (c 1.5 acetone); R_f in system A 0.42 (R_f for I in the same system 0.67). R_f in system B 0.92; E = 0.93. Calculated, %: C 66.0, H 6.22, N 9.62. $C_{24}H_{27}N_3O_5$. Found, %: C 65.74, H 6.23, N 10.30. b) To a solution of 0.07 g of IV in 0.7 ml of chloroform was added a solution of 0.05 g of III in 0.4 ml of chloroform. With periodic stirring, the mixture was kept at 20° for 6 h, after which the precipitate was filtered off and it was washed with chloroform. There was obtained 0.07 g of V having mp 186°, $[\alpha]_D^{20}$+ 5° (c 1.5, acetone). A mixed melting point with the material made by method a) gave no depression.

(–)-N'-Carbobenzoxy-L'-α'-glutamyl-L-α-methyltryptamine (VI). To a solution of 6.02 g of IV in 60 ml of chloroform was added a solution of 4.0 g of II in 29 ml of chloroform, and then a drop of acetic acid was added. The mixture was kept at 20° for 1 day and the gel which had formed was filtered off. After washing the precipitate with chloroform, 4.97 g of amorphous powder was obtained, which was dissolved in 50 ml of methanol and treated with 1 g of KU-2(H^+) ion-exchange resin to remove traces of II. After removal of the resin, the solvent was distilled off under vacuum and 4.20 g (42%) of VI was obtained in the form of an amorphous substance having $[\alpha]_D^{20}$ –16° (c 1.5, acetone), R_f 0.42 (A); 0.92 (B); E = 0.93. Calculated, %: C 66.00, H 6.22, N 9.62. $C_{24}H_{27}N_3O_5$. Found, %: C 66.02, H 6.18, N 9.95.

(+)-L'-α'-Glutamyl-D-α-methyltryptamine (glutramine) (VII). Compound V (5.9 g) was dissolved in 180 ml of methanol and was subjected to hydrogenolysis in a 0.5-liter autoclave at 45° and a pressure of 40 atm in the presence of 0.5 g of 10% Pd/C. Monitoring of the progress of reaction was effected by use of thin-layer chromatography in system A. After

*More accurately, 62%, calculating on D,L-methyltryptamine.

removal of the catalyst, the solution was treated with 1 g of activated charcoal and evaporated under vacuum to a volume of 10 ml. Dry ethyl acetate (150 ml) was added to the residue, and the suspension formed was allowed to stand overnight in a refrigerator. The precipitate was filtered off, washed with ethyl acetate, and dried. There was obtained 3.31 g (80%) of VII, mp 124-125°, $[\alpha]_D^{20}$ +41° (c 1.5, methanol), R_f 0.05 (A), 0.00 (B); E = 1.14. Calculated, %: C 63.36; H 6.98, N 13.83. $C_{16}H_{22}N_3O_3$. Found, %: C 63.40, H 7.02, N 13.83.

(–)-L'-α'-Glutamyl-L-α-methyltryptamine (VIII). A solution of 4.2 g of VI in 100 ml of methanol was subjected to hydrogenolysis in a 0.5-liter autoclave at 45° and 40 atm in the presence of 0.5 g of 10% Pd/C. Monitoring of the course of reaction was effected using thin-layer chromatography in system A. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in 0.15 liter of 15% aqueous ammonia. The solution was extracted three times with chloroform (50 ml). The aqueous layer was evaporated to dryness, toward the end, with addition of benzene. The glassy mass was ground with ether in a mortar. The ether was decanted. The residual mass was dried in air. There was obtained 1.84 g of VIII (62%), in the form of an amorphous powder. $[\alpha]_D^{20}$ –12° (c 1, methanol), R_f 0.05 (A), 0.00 (B); E = 1.14. Calculated, %: C 63.36, H 6.98, N 13.83. $C_{16}H_{21}N_3O_3$. Found, %: C 63.60, H 6.51, N 13.20.

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*As in Russian original; no date given – Consultants Bureau.