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SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF THE HYDROCHLORIDE OF d,l-α-TOCOPHEROL-γ-[N-ISONICOTINOYL]AMINOBUTYRATE

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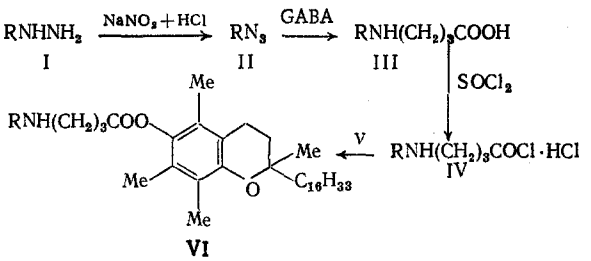
A compound which plays an important role in the functional processes of the brain and which has attracted the attention of clinicians is γ-aminobutyric acid (GABA) [6, 8, 9]. One of the drawbacks of this drug, however, is that when given orally it does not readily pass through the hematoencephalitic barrier (HEB) into the central nervous system. To overcome this disadvantage, and also to obtain compounds which not only have the properties of GABA, but also other desirable pharmacological properties, several approaches have been used, one of which consists of modifying its structure. This has led to the preparation of such compounds as piracetam, phenibut, baclofen, etc. Another approach involves the chemical coupling of the amino group of GABA with a biologically active compound [5, 14], which acts as a carrier to transport the GABA through the HEB; substances with new pharmacological properties may also be synthesized by this method.

Compounds investigated as carriers of GABA through the membranes of the cerebral capillaries included lipids [11], nicotinic [10], pantothenic [12], isonicotinic [4], and other acids, the esters of which with GABA in addition to exhibiting specific pharmacological action, to some extent or another participate in the regulation of the circulation of blood in the brain [2]. Thus, it was established that N-isonicotinyl-GABA (III) increased blood-circulation in the brain and lowered the tonus in the arterial system of the brain [7].

In the course of this work, we became interested in synthesizing and studying the pharmacological properties of d,l-α-tocopheryl-γ-[N-isonicotinoyl]aminobutyrate (VI), since vitamin E – a subsidiary component of the structure of N-isonicotinoyl-γ-aminobutyric acid (III) – plays an important role in the stabilization of the membrane [13].

Compound VI was obtained by the following scheme:



VI

R = Isonicotinoyl

The hydrazine of isonicotinic acid (I) with HNO_2 gave the azide (II), which was reacted with GABA in the presence of NaOH. From the sodium salt of N-isonicotinoyl-GABA (III) was obtained the hydrochloride of the acid chloride of N-isonicotinoyl-GABA (IV), which was condensed with freshly prepared d,l-α-tocopherol (V) in the presence of pyridine. The reaction product VI was purified, first by dissolving it in chloroform and treating this solution with the ion-exchange resin KU-2×8 in the H^+-form, and then by chromatography on a column of L40/100 silica gel (ChSSR). Treatment of VI with ethyl alcohol, saturated with HCl gas gave the hydrochloride of VI (VII), a pale-yellow crystalline substance readily soluble in alcohol.

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The composition and structures of compounds VI and VII were confirmed from elemental analysis, and IR, NMR, and UV spectral data.

The IR spectra of compounds VI and VII differ from that of the starting compound III, which contains absorption bands characteristic of its functional groups (λ_{max}, cm^{-1}): NH (3350), C=O (1720), COO^- (1610), CONH (1540), CONH (1290); the spectrum of III has no absorption at 1610 (COO^-), and the absorption at 1720 (C=O) is shifted to 1750 cm^{-1}, due to the formation of the ether.

In the NMR spectra of compounds VI and VII, there are peaks due to protons located as follows: the pyridine ring at C^2 and C^3 (9, 12, and 8.65 ppm, d 2H), the methylene groups of III at C_γ, of V at C^4, and of III at C_α (3.24 ppm, t, 2.65 ppm, t, and 2.10 ppm, d 2H), the methyl groups of V at C^5, C^7, C^8 (2.05 ppm, d 9H), the methylene groups of V at C^3, III at C_β, and the isoprene ring of V (1.75 ppm, t 2H; 1.75 ppm, m 2H, and 1.20 ppm, broad signal, 18H), the methyl groups of the isoprene ring of V (0.85 ppm, d 9H).

The UV spectra of compounds VI and VII have an absorption maximum at 276 nm with $E^{1\%}_{cm}$ equal to 85 and 117 respectively, approximately the same as for esters of V reported in the literature: d,l-α-tocopheryl-acetate (λ_{max} 285 nm, $E^{1\%}_{cm}$ 43.3) [1] and d,l-α-tocopherylphosphate (λ_{max} 286.5 nm, $E^{1\%}_{cm}$ 43.5) [3].

EXPERIMENTAL (CHEMICAL)

Reaction products were chromatographed on an ion-exchange resin column (KU-2×8 in the H^+ form), and on an L40/100 (ChSSR) silica gel column with the following solvent systems: $CHCl_3$ (system A) and $CHCl_3$–ether 9:1 (system B). TLC was carried out using Silufol UV-254 (ChSSR) plates and the solvent systems MeOH–water 1:1 (system C), and hexane–ether 1:2 (system D). Infrared spectra were taken on a UR-10 (GDR) spectrophotometer; compounds were prepared as mulls with mineral oil or in KBr pellets. UV spectra were recorded on a Hitachi EPS-3T (Japan) spectrophotometer at 240-350 nm. NMR spectra were recorded on a Brucker WM-250 (FRG) spectrometer with a working frequency of 250 MHz, using deuterated solvents $CDCl_3$, CD_3OD, and D_2O; internal standard TMS.

N-Isonicotinoyl-γ-aminobutyric Acid (III). To a vigorously stirred mixture of 22.60 g (0.165 mole) of the hydrazide of isonicotinic acid (I), 33.5 ml of HCl (d_4^{20} 1.18) and 17 ml of water at −5°C was added over a period of 2 hours a solution of 25.30 g (0.367 moles) of $NaNO_2$ in 33.7 ml of water, the solution brought to pH 7.0 by the addition of $NaCO_3$ and left to stand for 24 hours at 0-5°C. To the precipitated azide II, after decanting the mother liquor, was added a solution of 16.50 g (0.162 moles) of GABA in 16 ml of 1 N NaOH (pH 8.0-9.0) and the mixture stirred at 18-20°C for 24 hours. The solution was evaporated to dryness in vacuo (10-15 mm of mercury), the residual sodium salt of isonicotinoyl-GABA washed with MeOH (2×20 ml), then dissolved in 15 ml of water and acidified with 1 N HCl to pH 4.0. The mixture was maintained at 0-5°C for 12 hours. The precipitated material was separated, washed with water (2×15 ml) and dried in vacuo (0.1 mm) over P_2O_5 at 20°C to give 22 g (66.1%) of III with mp 166-167°C (decomp.). Found, %: C 57.60; H 5.80; N 13.48. $C_{10}H_{12}N_2O_3$. Calculated, %: C 57.69; H 5.81; N 13.45. UV spectrum, λ_{max}, nm ($E^{1\%}_{1cm}$) (MeOH): 271 (310). NMR spectrum (D_2O), δ, ppm: 8.74 d and 8.12 d (2H, H-2, 3); 3.24 t, 2.24 t, and 1.72 m (2H, H-α,β,γ). R_f 0.72 (system C).

d,l-α-Tocopheryl-γ-(N-isonicotinoyl)aminobutyrate (VI). A mixture of 5.75 g (0.028 mole) of III, 35 ml of dry toluene and 16.47 g (0.138 mole) of $SOCl_2$ was refluxed for 3 hours, and then evaporated to dryness in vacuo (10-15 mm mercury). To the residue was added a solution of 9.69 g (0.023 mole) of d,l-α-tocopherol (V) in 15 ml of dry pyridine, the mixture stirred for 3 hours in an atmosphere of N_2 at 50-60°C and then evaporated to dryness. The residue was dissolved in $CHCl_3$, passed through a column (50×100 mm) 2/3 filled with ion-exchange resin, and eluted with solvent system A. The crude product (11.83 g) of VI was chromatographed on a silica gel column (20×400 mm). The mixture was eluted with solvent system A, and VI with system B. The fraction containing the reaction product VI was collected and concentrated to dryness in vacuo (10-15 mm mercury) to give 5.07 g (36.3%) of VI. Found, %: C 75.40; H 9.72; N 4.50. $C_{39}H_{60}N_2O_4$. Calculated %: C 74.44; H 9.74; N 4.51. R_f 0.4 (system D).

Hydrochoride of d,l-α-Tocopheryl-γ-(N-isonicotinoyl)aminobutyrate (VII). To a solution of 5.07 g of VI in 3 ml of absolute alcohol was added 10 ml of alcohol, saturated with HCl gas. The precipitated material was separated and dried in vacuo (0.1 mm) at 20°C to give 4.83 g (90.0%) of VII with mp 84-86°C. Found %: C 71.53; H 9.54; Cl 5.41. $C_{39}H_{61}ClN_2O_4$. Calculated, %: C 71.26; H 9.35; Cl 5.39.

EXPERIMENTAL (PHARMACOLOGICAL)

Tests were conducted on cats under general anesthesia (urethane-chloralose) with artificially ventilated lungs. The effect of compound VII on the tonus of the cerebral blood vessels of the carotid and vertebral systems [8] was studied by resistography. The supply of blood to the brain through the artery which passes through the inside of the jaw was determined with an RKÉ-2 electromagnetic flow-meter. Simultaneously were recorded the EEG in the parietal region, the EKG, and the arterial pressure in the femoral artery. Recordings were made on a "Mingograf-81". Compound VII (10-20 mg/kg, intravenously) caused an increase in the blood supply to the brain (on average by 32%), which in some tests was observed immediately after administration of the drug, and in others after 5-10 minutes. The action of VII on the blood supply to the brain lasted for 10-20 minutes.

Compound VII, at this dosage, caused a lowering of the tonus of the cerebral blood vessels in the carotid arterial system by an average of 13.5%, and in the vertebral by 13.4%. Arterial pressure on average was decreased by 27%; in some tests, an initial rise in the level of the arterial pressure which gradually changed to a decrease was noted.

On studying the effect of VII on the neural regulation of the cerebral blood circulation, it was found that it weakened the constrictor reaction of the cerebral vessels, caused by the electrical simulation of the tibial nerve. In addition to this it was observed that the pressor reflex of the arterial pressure was depressed, and the EEG and EKG did not undergo any significant change.

Thus, compound VII increased the rate of cerebral blood flow by lowering the tonus of the vessels in both arterial systems in the brain, and weakened the reflector constrictor reaction of cerebral blood vessels. The cerebrovascular effect of compound VII was not as great as that of N-isonicotinoyl-GABA [7].

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