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ANTIFIBRINOLYTIC ACTIVITY OF NICOTINIC ACID CONJUGATES WITH 6-AMINOHEXANOIC, p-AMINOMETHYLBENZOIC, AND trans-4-AMINOMETHYLCYCLOHEXANECARBOXYLIC ACIDS

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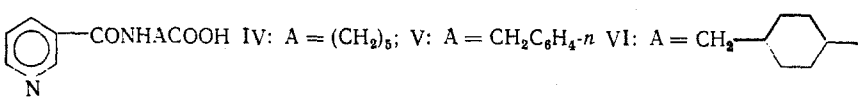
Studies of the antifibrinolytic activity of 6-aminohexanoic acid (I), p-aminomethylbenzoic acid (II), trans-4-aminomethylcyclohexanocarboxylic acid (III), and their analogs have been covered in a wide range of literature that offers data on biological action—structure relationships and a qualitative analyses of the contribution made by some functional groups to overall inhibitory activity [6]. It has been shown in particular that the presence of free amino and carboxyl groups separated by a distance of 0.7 nm is an important condition for the high level of antiproteolytic activity of this group of substances [9]. For the purpose of obtaining potential medicinal agents from compounds I-III, we undertook the synthesis of a number of their vitamin conjugates. Because of the vitamin presence in their molecular composition, we believed such compounds would be less toxic, exhibit a high degree of organotropy, as well as concomitant vitamin activity, and might also have better pharmacokinetic characteristics. That supposition has been recently justified by the production of vitamin conjugates with γ-aminobutyric acid [3, 7]. The present work cites results of studies on the antifibrinolytic activity of N-amide derivatives of nicotinic acid with substances of compounds I-III as compared to I. N-nicotinoyl-6-aminohexanoic acid (IV), p-aminomethylbenzoic acid (V), and trans-4-aminomethylcyclohexanecarboxylic acid (VI) were synthesized by the condensation of nicotinic acid chloroanhydride with salts of the corresponding acids in a water medium with a relatively low yield. This was apparently due to the partial hydrolysis of the chloroanhydride in the Schotten—Baumann reaction:



The structure of the substances obtained was confirmed by IR and UV spectra. The UV spectra of compounds IV-VI have maximum absorption bands at 259.5 nm that are due to the presence of a β-pyridylcarbonyl residue whereas the spectrum of compound V has a supplemental absorption band at 233 nm because of the disubstituted phenyl residue. The presence of intense absorption bands was observed in the IR spectrum of substances IV-VI in the region of 3300 (NH), 1710 (COOH), 1630-1640 (amide I), 1540-1550 (amide II), and 1590-1595 cm^{-1} (pyridine ring). Compounds IV-VI appear as crystalline substances that are slightly soluble in water and alcohol. The easily water-soluble sodium and potassium salts were obtained for our pharmacological study.

EXPERIMENTAL CHEMICAL

The IR spectra of compounds IV-VI were recorded in petroleum jelly on a Carl Zeiss UR-10 spectrophotometer (GDR). Compound purity was controlled by TLC on silufol UV-254

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TABLE 1. Comparative Test Results for the Antifibrinolytic Action of Nicotinic Acid Derivatives from in Vitro Experiments Using Different Plasmin Preparations

Compound	Lysis of fibrin plates, mm²								Number of tests
	standard		heated		% activator activity	Δ		% activator activity	
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		$\bar{x}$	$S_{\bar{x}}$		
				Novo plasmin					
Control	533,6	49,1	349,0	73,2	37,0	204,6	38,4	100	32
I	614,1	62,9	537,3*	57,6	12,5	76,8*	23,5	37,5	29
IV	476,0	60,0	380,6	77.5	20,1	95,4*	23,2	46,6	30
V	654,0**	63,8	516,6	87,4	21,0	137,4	23,7	67,2	29
VI	507,1**	25,9	414,7	33,5	18,2	92,4*	7,8	45,2	29
				Kiev Plant plasmin					
Control	465,3	24,6	257,3	20,7	44,7	208,0	17,5	100	109
I	448,3	29,0	338,9*	27,6	24,4	109,4*	9,5	52,6	115
IV	426,6	35,0	315,8	26,5	26,0	110,8*	14,8	53,3	106
V	461,6	34,4	340,0*	25,7	26,3	121,6*	18,6	58,5	93
VI	450,8	23,9	325,3*	22,7	24,2	125,5*	14,3	60,3	92

Note. One asterisk represents the t-criterion of the discrepancy reliability (≥2.0) relative to the series control. Two asterisks signify the t-criterion of reliability relative to the previous variant in the series.

plates (Czechoslovakia) in a 5:2:3 butanol—acetic acid—water system. UV irradiation was used to develop the substances on the chromatograms.

N-(Nicotinoyl)-6-aminohexanoic Acid (IV). A 1-g portion of nicotinic chloroanhydride chlorohydrate was added in small doses to a solution of 0.96 g of 6-aminohexanoic acid and 0.74 g of NaOH in 10 ml of water at 0-5°C. After the entire 1 g was added, the mixture was allowed to air-warm, with stirring, to 20°C after which 20 ml of water were added. The mixture was then acidified with concentrated HCl to pH 3.0-3.5. The resultant precipitate was filtered off, washed with water, and vacuum-dried. Yield 0.39 g (30.1%) of compound IV, mp 234-235.5°C (water). UV spectrum (water), λ_{max} (ε): 259.5 nm (3996). Found, %: C 61.39, H 7.09, N 12.28. $C_{12}H_{16}N_2O_3$. Calculated, %: C 61.01, H 6.78, N 11.86.

N-(Nicotinoyl)-p-aminomethylbenzoic Acid (V) was obtained in the same manner as IV from 5.89 g of p-aminomethylbenzoic acid. Yield 47%, mp 230-232°C (alcohol—water). UV spectrum (water), λ_{max}, nm (ε): 233 (18,343), 259.5 (4014). Found, %: C 65.22, H 4.74, N 11.32. $C_{14}H_{12}N_2O_3$. Calculated, %: C 65.62, H 4.72, N 10.93.

N-(Nicotinoyl)-trans-4-aminomethylcyclohexanecarboxylic Acid (VI) was obtained in the same manner as IV from 10.22 g of trans-4-aminomethylcyclohexanecarboxylic acid. Yield 40.5%, mp 214-216°C. UV spectrum (water), λ_{max} (ε): 259.5 nm (4187).

Sodium N-(Nicotinoyl)-6-aminohexanoate. A 4.5-g portion of compound IV was added in small doses to a solution of 2.02 g of sodium carbonate in 50 ml of water. The reaction mixture was then stirred at 40°C until the acid was completely dissolved. The precipitate was then separated, the filtrate was evaporated, and the residue was dried in a vacuum. Yield 4.87 g (99%) of the sodium salt. Na and K salts of compounds IV-VI were obtained in a similar fashion.

TABLE 2. Comparative Test Results of the Antifibrinolytic Action of Nicotinic Acid Derivatives in in Vivo Experiments

Compound	Lysis of fibrin plates, mm²								Number of tests
	standard		heated		% activator activity	Δ		% of activator activity	
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		$\bar{x}$	$S_{\bar{x}}$		
Control	551,7	31,5	190,6	26,1	65,5	361,1	41,7	100	84
I	460,0*	24,9	303,8*	42,7	34,0	164,6*	38,6	45,6	60
IV	408,1*	21,9	215,7	21,4	47,1	192,4*	38,4	53,3	94
V	460,4*	24,1	224,5	38,1	49,9	229,0*	30,8	63,4	64
VI	472,4*	15,9	302,2*	21,7	36,0	170,2*	17,7	47,1	50

*t-criterion of discrepancy reliability (≥2.0) relative to the control.

EXPERIMENTAL BIOLOGICAL

Enzymatic activity was assayed by the Astrup method [10] on stabilized fibrin plates prepared by the P. S. Gribauskas and A. P. Markunene modification [2]. A portion of the plates were heated at 86°C for 30 min. Total plasminogen activity and fundamental plasmin fibrinolytic activity were assayed on standard plates. The heated plates were used to assay plasmin fibrinolytic activity. The content of the plasminogen activators was determined by the difference in the lysis zones on the standard and heated fibrin plates. The antiplasmin activity of compounds I, IV-VI was examined at a concentration of 10^{-2} M. Plasmin specimens used for this assay were made by the Novo Company (Denmark) and the Kiev Bacteriological Preparations Plant. The concentration of the plasmin in the incubation mixture was 2 mg/ml. Solutions of the test preparations were preincubated with plasmin for 30 min at 37°C and a pH of 7.8. During that time 0.03 ml of the incubation mixture were placed on four points of the fibrin plate. The size of the lysis was measured 24 h after incubation at 37°C.

The effect of the compounds on blood coagulation and fibrinolytic activity was tested in vivo on white male rats of a single breed, weighing 220-240 g. Compound I was injected subcutaneously at a dose of 10 mg/100 g of animal weight 2-3 min after an IV injection of thrombin at a dose of 40 ED per 250 g of animal weight in 1 ml of a NaCl physiological solution in order to produce a fibrinolytic condition [5]. The test blood was taken from the jugular vein and was immediately stabilized by a 3.8% solution of sodium citrate at a 1:9 ratio. The citrate plasma was tested for fibrinolytic activity on heated and standard fibrin plates [10]. Similar tests were conducted for compounds IV-VI whose therapeutic doses were 45.1, 13.4, and 13.76 mg/ml, respectively, and equimolar to compound I. In vivo tests were conducted for compound I (5% solution for IV injections of a preparation made by the N. A. Semashko Moscow Pharmaceutical Chemical Plant). Compound I made by the Chemapol firm of Czechoslovakia was used for in vitro experiments.

Two different samples of plasmin were used in the first-stage tests on standard and heated fibrin plates in order to differentiate the antiproteolytic action of the examined compounds I, IV-VI on the activation system on plasminogen (antiactivator activity) and the process of fibrinolysis itself. Table 1 presents the results of our study of the fibrinolytic activity of the plasmin preparation manufactured by the Novo firm. As one can see from the cited data, the system under examination had a pronounced activator activity which accounted for 37% of all fibrinolytic activity. The introduction of compounds I, IV, V, and VI at a concentration of 10^{-2} M into the incubation medium resulted in a marked 12-21% decrease in activator activity. An analysis of antiactivator activity showed that compounds I, IV, and VI had a pronounced antiplasmin action of almost identical intensity. No reliable changes in activator activity were detected upon the introduction of preparation V.

Table 1 shows similar test results for the compounds where the plasmin preparation used was manufactured by the Kiev Bacteriological Preparations Plant.

Simulation of the fibrinolytic system which employed this plasmin preparation indicated that the activator activity was 44.7% of the total lysis zone area in the fibrin plate. Upon the introduction of compounds I, IV-VI this index decreased almost uniformly in all experimental variants to 24.2-26.3%. Our computation of antiactivator activity showed a practically identical reliable almost twofold decrease in plate lysis in all cases. When inhibitors were employed the activator activity decreased to 52-60% of the control level.

Table 2 gives the in vivo test results for the compounds under study. In view of the considerable stability of the fibrinolytic system in the physiologically normal animals, and particularly in the white rats [1], we used the fibrinolysis activation model by means of an intravascular injection of thrombin. The examination of blood taken IV showed that the plasma of these animals exhibited a rather pronounced ability to lyse fibrin plates. Moreover, the difference in the lysis on the standard and heated plates would indicate that the activator activity in the control animals accounts for 65.5% of the total lysis zone. The latter markedly decreased (by 1.3-1.8 times) when the fibrinolysin inhibitors were administered subcutaneously. As can be seen from the computed data on the activator activity of the test compounds, the administration of compounds I, IV-VI resulted in a significant decrease in the fibrinolysin zone which corresponded to 45.6%, 53.3%, 63.4%, and 47.1% of the control level, respectively. One can conclude from the obtained data that compounds IV-VI, when administered at a concentration isomolar to compound I, effectively inhibit the fibrinolytic activity in white male rats induced by an IV injection of thrombin. It is important to emphasize that substances I and IV have very similar degrees of antiactivator action.

The stability of IV during an incubation period of up to 60 min in a Barbital sodium-Barbital buffer at 37°C was examined under fibrinogenolysis conditions obtained by the use of a domestically manufactured fibrinolysin preparation. Paper chromatography in 5:2:3 butanol–acetic acid–water system of the incubation mixture components indicated the absence of the component with R_f 0.43 which corresponded to I. Consequently, antiplasmin activity was exhibited in vitro by compound IV, but not by its decomposition products.

The reaction between the ω-aminocarboxylic acids, particularly 6-aminohexanoic acid, and the lysine-bonding sites of the five triple-hinged structures of the plasminogen A-chain, play a decisive role in the underlying mechanism of the aminocarboxylic acid activity [4, 8]. In spite of the variable affinity of the lysine-bonding sectors of I, it is assumed that the protonated imidazole nitrogen of histidine interacts with the carboxyl group of the amino acid I whereas its protonated ε-amino function interacts with the carboxyl group of the polypeptide chain which most probably belongs to aspartic acid [8, 11]. Saturation of the lysine-bonding sectors leads to conformational plasminogen conversions that are secured by the "pockets" of the hinged structures held by the disulfide bonds [8, 10, 12].

In view of the in vivo antiplasmin activity of compounds IV-VI and their inhibition of thrombin-induced fibrinolysis that is practically identical to that of compound I, one should take into consideration the ability of compound I to inhibit strongly the reaction between plasmin and plasminogen as well as its ability to increase the dissociation of the plasminogen–fibrin complex [8]. It is in fact that mechanism which explains the antiplasmin activity of compound I and its analogs in in vivo experiments.

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SYNTHESIS, TRANSFORMATIONS, AND BIOLOGICAL PROPERTIES OF 7,8-DISUBSTITUTED XANTHINES

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Derivatives of xanthine are valuable medicinal compounds having, in particular, pronounced bronchiolytic properties [1]. Theophylline and euphylline, used for prophylaxis and curing of bronchospasms in bronchial asthma [7], block phosphodiesterase in lung tissues and cause the accumulation of cyclic adenosine triphosphate [9].

Based on the above considerations, and in view of the urgency of finding new broncholytic preparations, we investigated the synthesis of several 7,8-disubstituted xanthines in order to study their broncholytic activity.

7-(2,3-Dihydroxypropyl)-8-bromotheophylline (II) was obtained by the reaction of 8-bromotheophylline (I) with glycidol in the presence of catalytic quantities of pyridine. The previously described [3, 8] 6,8-dimethyl-2-hydroxymethyloxazolino[3,2-f]xanthine (III) was obtained by the action of an equimolar amount of an alcoholic solution of alkali on the dihydroxy derivative II.

The derivatives of 7-(2,3-dihydroxypropyl)-8-aminotheophylline (IV-VIII) were synthesized by aminolysis of compounds II and III. It should be noted that the preparation of amines from oxazolinoxanthine III by cleaving the oxazoline ring proceeds much more easily than by substitution of the bromine atom in the bromo derivative II.

8-Bromotheophyllinyl-7-acetaldehyde (IX) was obtained by glycolic cleaving of compound II by periodic acid. In the case of the oxidation of amino alcohols, for example VII and VIII, the amino-aldehydes (X, XI) could not be isolated, since they undergo cyclization into the derivatives of imidazo[1,2-f]xanthine (XIII, XV). To confirm the above scheme of transformations, we prepared a series of derivatives of imidazo[1,2-f]xanthine XII-XV by the reaction of aldehyde IX with primary amines.

The structure of the synthesized compounds II-XV was confirmed by the data of IR and PMR spectroscopy. (See Scheme at top of next page.)

EXPERIMENTAL CHEMICAL

The IR spectra were run on an UR-20 spectrophotometer (GDR) in KBr tablets and in mineral oil. The PMR spectra were obtained on a "Tesla-487C" spectrometer (80 MHz) at 20°C, using HMDS as internal standard.

7-(2,3-Dihydroxypropyl)-8-bromotheophylline (II). A mixture of 25.9 g (0.1 mole) of 8-bromotheophylline (I), 8.15 g (0.11 mole) of glycidol, and 1.5 ml of pyridine in 150 ml of propanol was heated at the boiling point for 90 min. It was then cooled and the precipitate was filtered. The data on the compounds synthesized are given in Table 1.

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