SYNTHESIS AND BIOLOGICAL ACTIVITY OF N-SUBSTITUTED 1,4-PERHYDROTHIAZIN-1-OXIDES

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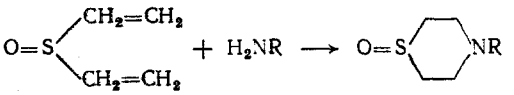
Proteolytic enzymes, which play an essential role in important biological processes (protein volume, blood coagulation, regulation of blood pressure), also enter into the arsenal of effective materials for antithrombosis therapy [3]. Hydrolysis of polypeptide chains can be brought about with synthetic catalysts acting as enzymes [6] and therefore prospects seemed promising for the imitation of the action of plasmin by the use of specific chemical compounds, for which we selected N-substituted 1,4-perhydrothiazin-1-oxides. Accounts of their synthesis from primary amines are very limited [1, 5].

In the present communication we report the synthesis and a study of the biological properties of new N-substituted 1,4-perhydrothiazin-1-oxides (IIa-c) and their salts (IIIa-d, IVa, b), and also on the possibility of their use for pharmacocorrective hemostasis. We first studied the reaction of divinylsulfoxide, for which we had earlier developed a convenient and effective synthesis [4] with the series of carbonfunctionalized primary amines I(a-c).

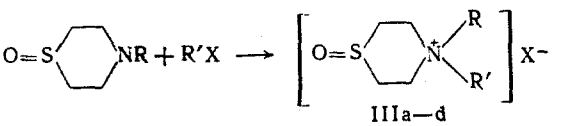


Ic-c IIa-c

IIa: $R = (CH_2)_2OH$; IIb: $R = (CH_2)_5COOH$; IIc: $R = (CH_2)_2OCH{=}GH_2$.

The reaction of these reagents easily took place in water or in alcohol at 55-60°C. The yield of intermediate products IIa-c was 80-90%.

Reaction of the N-substituted perhydrothiazine oxides IIa and IIc with alkyl monohalides gave the quaternary ammonium salts IIIa-d in quantitative yield.



IIIa—d

IIIa: $R = (CH_2)_2OH$, $R' = C_2H_5$, $X = Br$; IIIb: $R = (CH_2)_2{-}OCH{=}CH_2$, $R' = = CH_2C_6H_5$, $X = Cl$; IIIc: $R' = CH_3$, $R = (CH_2)_2OCH{=}CH_2$, $X = I$; IIId: $R = = (CH_2)_2OCH{=}CH_2$, $R' = CH_2CH{=}CH_2$, $X{=}Br$.

The reaction proceeded easily in nitromethane, acetonitrile, or ethanol at 80°C in 5 h (for compound IIIa) or at 20°C for 10 h (for IIIb-d).

The treatment of IIa with hydrogen chloride or boron trifluoride etherate (in water) under mild conditions (5-20°C) gave the corresponding salts IVa and IVb.

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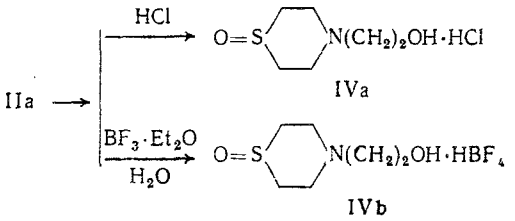

The composition and structure of the compounds prepared were confirmed by elemental analysis (Table 1) and by NMR (^1H and ^{13}C) and IR spectroscopy (Table 2).

EXPERIMENTAL

Chemistry

NMR spectra of the above compounds dissolved in CCl_4 or D_2O were obtained on a Kh-90 Q (JEOL) spectrometer (with internal standards of HMDS or DSS, respectively: resonance frequency for ^1H = 89.55 MHz, and for ^{13}C = 22.49 MHz). IR spectra were recorded with a Specord-75 spectrophotometer as a thin film for compound IIa, and as KBr disks for the remaining compounds.

4-(2-Hydroxyethyl)-1,4-perhydrothiazin-1-oxide (IIa). To 12.24 g (0.12 mole) of divinylsulfoxide dissolved in 15 ml of ethanol were added dropwise with stirring 6.11 g (0.1 mole) of monoethanolamine (Ia). The reaction mixture was heated at 55-60°C for 5-6 h. Precipitation with ether from the ethanol solution gave 14.5 g (89%) of IIa.

4-(5-Carboxypentyl)-1,4-perhydrothiazin-1-oxide (IIb). To 4.48 g (0.044 mole) of divinylsulfoxide was added dropwise a solution of 5.2 g (0.04 mole) of 6-aminohexanoic acid (Ib) in 10 ml of water. The mixture was heated for 6-7 h at 60°C and precipitated from the ethanol with ether to give 7.6 g (82%) of white crystalline IIb. An analogous preparation gave IIc.

4-Ethyl-4-(2-hydroxyethyl)-1-oxo-1,4-perhydrothiazium Bromide (IIIa). To a solution of 3.26 g (0.02 mole) of IIa in 6 ml of nitromethane were added with stirring 3.27 g (0.03 mole) of ethyl bromide in 4 ml of the same solvent. The reaction mixture was heated for 5 h at 80°C and isolated by precipitation from ethanol with ether to give 5 g (92%) of IIIa.

Benzyl-4-vinyloxyethyl-1-oxo-1,4-perhydrothiazinium Chloride (IIIb). To a solution of 1 g (0.005 mole) of thiazine oxide IIc in 10 ml of nitromethane were added dropwise 0.63 g (0.005 mole) of benzyl chloride and the reaction mixture was kept 10-12 h at 20°C to give 1.62 g (97%) of white crystalline IIIb. Compounds IIIc and IIId were obtained in an analogous fashion.

4-(2-Hydroxyethyl)-1-oxo-1,4-perhydrothiazium Chloride (IVa). Into a solution of 1 g (0.006 mole) of thiazine oxide IIa in 20 ml of anhydrous methanol at 20°C was passed dry hydrogen chloride for 1 h. Distillation of the solvent gave 1.05 g (86.1%) of IVa.

4-(2-Hydroxyethyl)-1-oxo-1,4-perhydrothiazinium Hydrotetrafluoroborate (IVb). To a solution of 1 g (0.006 mole) of thiazine oxide IIa in 20 ml of water at 5-15°C was added 1 g (0.007 mole) of borontrifluoride etherate. The solvent was removed immediately to give 1.1 g (71.4%) of IVb.

Pharmacology

The studies were carried out with rabbit blood plasma taken from the end of the ear veins and stabilized (9:1) with sodium citrate solution. The citrated plasma was incubated for 3 min at 37°C in the presence of the above compounds (concentration range = 5000-30 μg/ml) and then the recalcification time was determined [2] for the appearance of a direct anticoagulant effect. The lytic activity of materials IIa-c, IIIa-d, IVa, and IVb was studied with fibrin stabilized by factor XIIIa by the method of B. A. Kudryashov [2]. The results were recorded 2.24, and 48 h after application of solutions of compounds II-IV to the fibrin clot.

Incubation of these materials in blood plasma in doses pof 5-2.5μg/ml with added Ca^{2+} produced a moderate acceleration of the coagulation reaction: for IIb and IIIa, by 10-15%; for IIc, by 15-18%; for IVb and IVa, by 15-20%; for IIa, by 17-20%, and for IIIb-d, by 20-24%. Lower concentrations of compounds II-IV (1000-30 μg/ml) did not show a significant effect. Identical results were obtained upon introduction of the materials into native blood for automatic recording of the hemocoagulation process by thromboelastogram.

TABLE 1. N-Substituted 1,4-Perhydrothiazines and Their Salts

Compound	Yield, %	bp, °C (mm Hg); mp, °C	Found, %					Empirical formula	Calculated, %				
			C	H	Hal	N	S		C	H	Hal	N	S
IIa	89	165 ($5 \cdot 10^{-2}$)	44,23	8,04	—	8,58	19,63	$C_6H_{13}NO_2S$	44,17	7,98	—	8,58	19,63
IIb	82	110	51,30	8,24	—	6,35	13,78	$C_{10}H_{19}NO_3S$	51,46	8,21	—	6,01	13,75
IIIa	92	138	...	...	29,37	5,33	12,80	$C_8H_{18}BrNO_2N$	...	...	30,94	5,40	12,42
IIIb	97	143	...	...	11,57	4,33	10,10	$C_{15}H_{22}ClNO_2S$	...	...	11,24	4,43	10,13
IIIc	95,7	158	...	...	38,61	4,83	9,85	$C_9H_{18}INO_2S$	...	...	38,70	4,23	9,67
IIId	93	146	...	...	25,76	4,47	10,55	$C_{11}H_{20}BrNO_2S$	...	...	27,17	4,76	10,88
IVa	86	168	...	...	17,65	6,94	16,23	$C_6H_{14}ClNO_2S$	—	—	17,79	7,00	16,04
IVb	71,4	203	28,69	5,56	29,95	5,58	12,67	$C_8H_{14}BF_4NO_2S$	28,71	5,62	30,27	5,58	12,77

TABLE 2. Spectral Characteristics of Compounds II-IV

Compound	^{1}H NMR Spectrum, δ, ppm				^{13}C NMR Spectrum, δ, ppm								IR Spectrum, ν, cm^{-1}
	=CH	$=CH_2$	CH_2O	NCH_2	=CH	$=CH_2$	CH_2O	CH_2N	CH_2CH_2	CH_2OH	CH_2CH_2OH	SCH_2	
IIa	—	—	3,65 m*	—	—	—	—	—	—	—	—	—	3370—3400 (OH), 1020—1030 (S=O)**
IIIb	6,61 dd	4,35 m	—	—	149,84***	88,20	60,46	47,29	38,19	—	—	—	3020, 1260 (HC=C), 1030 (S=O)
IIIc	6,53 dd	—	—	3,35 s	148,94	87,73	59,70	49,90	37,78; 37,51	—	—	—	3020 (HC=), 1640, 1620 (C=O) 1060 (S=O)
IIId	6,38 dd	4,12 m	—	—	149,00	87,67*4	59,71	47,31	37,51	—	—	—	3050, 3028 (HC=) 1660, 1632 (C=C), 1060, 1040 (S=O)
IVe	—	—	—	—	—	—	—	39,94	—	57,06	53,49	41,30	3220 (OH), 2700 (NH), 1060—1085 (S=O)
IVd	—	—	—	—	—	—	—	40,68	—	57,75	54,17	42,04	3570 (OH), 2670 (NH), 1030 (S=O)

*All of the remaining methylene protons gave a poorly-resolved multiplet centered at 2.8 ppm.
*For IIb: 1700 (C=O), 1030 cm^{-1} (S=O); and for IIIa: 1030-1040 cm^{-1} (S=O).
‡C_6H_5: ^{1}H NMR: 7.60 ppm. ^{13}C NMR: C(1) 124.76, C(3, 5) 128.77, C(2, 6) 132.84, C(1, 4) 130.61 ppm, all singlets.
**For the $CH_2CH{=}CH_2$ group: 121.42 ppm (=CH), and 129.49 ppm ($=CH_2$).

TABLE 3. Alteration of Nonenzymatic Lytic Activity of N-Substituted 1,4-Perhydrothiazin-1-oxides and Their Salts

Compound	Concentration used, %		
	1	0.5	0,25
IIa	105±7,8	64±2.5	36±4,2
IIIa	39±4,3	0	0
IIIc	52±5,6	0	0
IIId	26±3,4	0	0
NaCl	0	0	0

The lytic action of our synthesized compounds on fibrin was studied under conditions *in vitro*. Compounds IIa, IIIa, c, and d increased the total and nonenzymatic fibrinolytic activity of blood plasma. In this case, the maximum nonenzymatic activity, measured by the area of lysis of nonstabilized fibrin plastin, increases to 105 ± 7.8 mm^2 for compound IIa (Table 3).

Studies conducted on the pure fibrin monomer f-dez-AABB, free from contamination by plasminogen, the polymerization of which was conducted in neutral (pH 7.4) medium at 37°C for 1 h, showed that a 1% solution of compound IIa lysed nonstabilized fibrin by 28.7 ± 4.2% in 2 h, and IIIa, c, and d insignificantly (10-13%) lysed clots only after 48 h.

The capability of the synthesized compounds to dissolve fibrin stabilized by factor XIIIa was confirmed on a fibrinogen preparation, preliminarily freed from plasminogen on lysine-pharose. It was established that compound IIa (1% solution) lysed 49.3 ± 3.9% of fibrin in 2 h, and in 24 h gave 100% lysis of a fibrin clot. Compounds IIIa, c, and d (1% solutions) produced fibrin lysis in 48 h: IIIa, by 32.5 ± 2.6%; IIIc, by 30.3 ± 3.5%; and IIId, by 44.1 ± 5.7%.

Thus, this investigation has shown that N-substituted 1,4-perhydrothiazin-1-oxides and their salts lyse fibrin stabilized by factor XIIIa.

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