BINUCLEAR DERIVATIVES OF PHENYLMERCAPTOACETIC ACID AND THEIR TRIS(2-HYDROXYETHYL)AMMONIUM SALTS. EFFECT ON THE FUNCTIONAL ACTIVITY OF THROMBOCYTES

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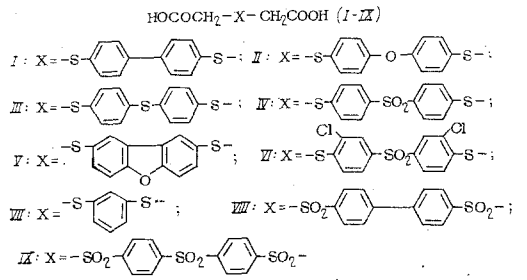
The development of methods for the medicinal prophylaxis and treatment of thromboembolic diseases is extremely urgent. The functional state of thrombocytes (adhesiveness, aggregation, etc.) plays an important role in the formation of structural thrombi in vessels with a fast blood flow [1]. A promising direction in the creation of preparations that regulate hemostasis is the synthesis of substances that inhibit the aggregation of thrombocytes [2].

The ability to decrease the aggregation of thrombocytes has been observed for many medicinal preparations [3], particularly for hypocholesterolemic agents (clofibrate, cetamiphen, etc.). In addition to decreasing the percentage of cholesterol in the blood, they decrease the amount of fibrinogen and the tendency for thromboses and intensify the activity of anticoagulants [3, 4].

Moreover, the dominating significance of the oxidation of arachidonic acid in the aggregation of thrombocytes has recently been revealed [5-7]. It has been established that the use of inhibitors of the free-radical oxidation of lipids is promising for the treatment of disruptions of the aggregation of thrombocytes. We have synthesized the following phenylmercaptoacetic acid derivatives:



Dibasic acids I-IX were converted to the previously unknown tris(2-hydroxyethyl)ammonium salts (X-XVI). Their effect on the functional activity of thrombocytes was studied. The latter are similar in structure to the hypocholesterolemic preparations named above and contain 2-hydroxyalkyl groups, which are responsible for the activity of a number of antiaggregants of thrombocytes [8-11] and phospholipids [12].

The reaction of the corresponding aromatic dithiols with monochloroacetic acid in an aqueous solution of an equivalent amount of potassium hydroxide was used for the synthesis of acids I-IX:

$$H{-}X{-}H + 2ClCH_2COOH + 2KOH \longrightarrow HOOCCH_2{-}X{-}CH_2COOH + 2KCl$$

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TABLE 1. Dibasic Organylmercapto- and Organylsulfonylacetic Acids (I-IX)

Compound	Yield, %	mp, °C	Found			Empirical formula	Calculated		
			C	H	S		C	H	S
I	90	245—246	57,89	4,42	19,04	$C_{16}H_{14}O_4S_2$	57,48	4,19	19,16
II	92	165—166	55,08	3,82	18,36	$C_{16}H_{14}O_5S_2$	54,84	4,00	18,29
III	93	194—195	52,53	3,74	26,10	$C_{16}H_{14}O_4S_3$	52,46	3,82	26,23
IV	91	187—188	48,27	3,50	24,00	$C_{16}H_{14}O_6S_3$	48,24	3,52	24,12
V	88	230—231	55,30	3,53	18,40	$C_{16}H_{12}O_5S_2$	55,17	3,45	18,39
VI*	85	183—185	—	—	20,41	$C_{16}H_{12}Cl_2O_6S_3$	—	—	20,58
VII	85	131—131,5	46,30	3,63	24,53	$C_{10}H_{10}O_4S_2$	46,51	3,88	24,80
VIII	92	280—282	48,51	3,63	16,32	$C_{16}H_{14}O_8S_2$	48,24	3,52	16,08
IX	87	279—280	41,27	2,98	21,03	$C_{16}H_{14}O_{10}S_3$	41,56	3,03	20,78

*Found: Cl 14.98%. Calculated: Cl 15.17%.

The yields, melting points, and results of elementary analysis of the synthesized acids I-IX are presented in Table 1. Compounds VIII and IX were obtained in 87-92% yields by oxidation of acids I and III, respectively, with 30% hydrogen peroxide in glacial acetic acid at 110°C for 2 h.

All of the synthesized acids were white, crystalline, odorless substances that are stable during storage, insoluble in water, slightly soluble in alcohol, and quite soluble in dimethyl sulfoxide (DMSO).

The IR spectra of acids I-VII contain absorption bands at 1690-1720 and 2500-2740 cm^{-1}, which correspond to the stretching vibrations of the C=O and OH groups in the carboxylic acids. The bands at 1165-1170 and 1315-1330 cm^{-1} in the IR spectra of VIII and IX correspond to asymmetrical and symmetrical vibrations of the sulfonyl group, and the absorption band of the carbonyl group is shifted 15-20 cm^{-1} to the high-frequency region as compared with the spectra of acids I-IV and VII.

The tris(2-hydroxyethyl)ammonium salts (Table 2) of acids I-IX were obtained by reaction of the acids with triethanolamine in alcohol.

Only mono[tris(2-hydroxyethyl)ammonium] salt XVI is formed in the reaction of acid VI even with a large excess of triethanolamine. The absorption bands of the $C(O)O^-$ groups in the IR spectra of salts X-XVI are shifted 80-100 cm^{-1} to the low-frequency region as compared with the spectra of the acids. The series of unresolved bands at 2400-2800 cm^{-1} corresponds to the absorption of the $\overset{+}{N}H$ groups; an absorption band of a hydroxy group is observed at 3400 cm^{-1}.

Tris(2-hydroxyethyl)ammonium salts X-XVI are white, crystalline, odorless substances that are stable during storage and soluble in water and alcohol.

Dibasic acids I-IX are characterized by two dissociation constants. Thus the pK_a values for acid II are 5.53 and 5.84, as compared with 5.01 and 6.06 for acid IV. Thus, these acids are weaker than benzoic (pK_a = 4.17 [13]) and phenylacetic (pK_a = 4.31 [13]) acids.

The second dissociation constants are approximately the same as those for monobasic arylmercaptoacetic acids for which X = $C_6H_4SCH_2COOH$ (pK_a = 5.8-6.0 [13]).

The pK_a value of the first degree of dissociation is lower than the pK_a value of the second degree of dissociation and decreases substantially in the case of oxidation of the side S atoms to sulfonyl groups. This intensification of the acidic properties of the dibasic acids is similar to that observed in the series of monobasic acids on passing from arylmercapto- to arylsulfonylacetic acids (pK_a = 4.7). The pH values of aqueous solutions of salts of acids I-V with triethanolamine, which is a weak base (pK_a = 7.8), depend only slightly on the structure of the acid and range from 6.4 to 6.6; the pH values of organylsulfonylacetic acids range from 5.80 to 5.85.

EXPERIMENTAL

4,4'-Bis(carboxymethylthio)diphenyl Sulfone (IV). A 0.1-mole sample of 4,4'-dimercaptodiphenyl sulfone, 0.1 mole of monochloroacetic acid, and 0.1 mole of potassium hydroxide were stirred in 100 ml of water at 100°C for 3 h, after which the mixture was cooled, acidified

TABLE 2. Tris(2-hydroxyethyl)ammonium Salts (X-XVI) of Binuclear Derivatives of Organylmercaptoacetic Acids

Compound	Starting acid	Yield, %	mp, °C	Found				Empirical formula	Calculated			
				C	H	N	S		C	H	N	S
X	I	91	155—157	54,80	6,86	4,19	9,70	$C_{28}H_{44}N_2O_{10}S_2$	54,86	6,76	4,25	9,75
XI	II	75	66—68	51,70	6,56	4,39	10,00	$C_{28}H_{44}N_2O_{11}S$	51,84	6,83	4,32	9,88
XII	III	73	85—87	50,50	6,60	4,27	14,39	$C_{28}H_{44}N_2O_{10}S_3$	50,58	6,67	4,21	14,46
XIII	IV	80	84—86	47,92	6,20	4,38	13,81	$C_{28}H_{44}N_2O_{12}S_3$	48,26	6,36	4,02	13,80
XIV	VII	72	53—54	47,74	7,25	4,93	10,96	$C_{22}H_{40}N_2O_{15}S_2$	47,42	7,24	5,03	11,52
XV	V	86	151,5—153	52,30	6,36	4,30	9,20	$C_{28}H_{42}N_2O_{11}S_2$	52,06	6,55	4,31	9,91
XVI*	VI	83	157—162	—	—	2,26	—		—	—	2,27	—

*Found, %: Cl 11.50%. Calculated, %: Cl 11.50%.

Note: Salts X-XV contain two molecules of the base per molecule of the acid, whereas XVI contains one molecule of the base per molecule of the acid.

TABLE 3. Effect of Tris(2-hydroxyethyl)ammonium Salts X-XVI in the Maximally Effective Dose on the Activity of Thrombocytes

Compound	Electrokinetic potential*			Aggregation of the thromocytes†		
	effective dose, M	$M_{e,c} \pm m_{e,c}$ ‡	T	effective dose, M	$M_{e,c} \pm m_{e,c}$ ‡	T
X	10^{-5}	20,5±6,1	3,36	10^{-3}	23,6±5,9	4,00
XI	10^{-3}	13,7±7,2	1,90	10^{-4}	19,7±7,1	2,77
XII	10^{-5}	15,4±6,0	2,57	10^{-4}	15,2±6,2	2,45
XIII	10^{-3}	20,7±6,2	3,34	10^{-4}	17,3±7,4	2,34
XIV	10^{-5}	12,1±7,6	1,59	10^{-4}	25,4±7,2	3,53
XV	10^{-4}	17,2±5,4	3,18	10^{-5}	23,5±6,4	3,67
XVI	10^{-6}	19,3±7,5	2,57	10^{-6}	17,6±5,7	3,09

*In percent with respect to intact thrombocytes (0.76 ± 0.04 mm·sec^{-1}·cm^{-1}).

†The inhibiting effect in percent with respect to the control, viz., ADP in a final concentration of 200 μg/ml.

‡The $M_{e,c}$ value is the difference between the experimental and control sets, and the $m_{e,c}$ value is the error in the difference.

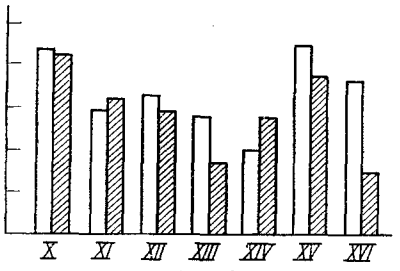

Fig. 1. Total biological activity of salts X-XVI expressed in the logarithm of the area under the dose–effect curve over the concentration range 10^{-3} to 10^{-12} M. The shaded columns pertain to inhibition of the aggregation of the thrombocytes, and the unshaded columns pertain to the change in the electrokinetic potential of the thrombocytes.

with concentrated HCl, and allowed to stand at 0-5°C for 8 h. The precipitated crystals were removed by suction filtration, washed with water, and dried to give 35.5 g of acid IV with mp 187-188°C. Acids I-III, V, and VIII were similarly obtained (see Table 1).

4,4'-Bis(carboxymethylsulfonyl)diphenyl Sulfone (IX). A 0.1-mole sample of 4,4'-bis(carboxymethylthio)diphenyl sulfide (III) was dissolved in 100 ml of glacial acetic acid, 68 ml of 30% hydrogen peroxide was added dropwise, and the mixture was heated at 100°C for 2-3 h. It was then cooled and diluted with water, and the precipitated crystals were removed by suction filtration and dried *in vacuo*. The yield of acid IX was 40.2 g (87%). Acid VIII was similarly obtained (see Table 1).

4-(4'-Carboxymethylthiophenyl)phenylmercaptoacetic Acid Bis[tris(2-hydroxyethyl)]ammonium Salt (X). A 0.01-mole sample of acid I was mixed with 40 ml of ethanol, a solution of 0.02 mole of triethanolamine in 10 ml of alcohol was added, and the mixture was heated to the boiling point and maintained at this temperature for 0.5-1 h until the acid had dissolved completely. The reaction mixture was cooled, and the precipitated crystals were removed by filtration and dried *in vacuo* to give 5.7 g (91%) of product. Salts XI-XVI were similarly obtained (see Table 2).

EXPERIMENTAL PHARMACOLOGY

Screening of potential inhibitors of the aggregation of thrombocytes was accomplished by measurements of the electrokinetic potential of blood cells by the Abramson method in the Kharamonenko modification [14] over X-XVI concentrations ranging from 10^{-3} to 10^{-12} M.

Normal citrate plasma enriched with thrombocytes was incubated at 37°C for 15 min in the presence of the investigated compounds. The decrease in the optical density (DOD) in percent with respect to a control was recorded before and after incubation. Adenosine diphosphate (ADP) in a concentration of 200 μg/ml was added to the samples after incubation.

The surface charge was determined from the rate of migration of the blood cells in an electrical field, and the increase in charge relative to the control (in percent) was calculated.

The experiments were carried out on rabbit blood plasma taken from the peripheral vein of the ear; blood platelets were obtained by the Rudchenko method [15]. Blood stabilized in a ratio of 9:1 with a solution of sodium citrate was centrifuged at 1000 rpm for 10 min.

The change in the functional activity of the thrombocytes in the presence of salts X-XVI is presented in Table 3 and Fig. 1.

A study of the functional activity of thrombocytes under the influence of salts X-XVI showed that they all increase the electrokinetic potential of blood cells by 13-23% and moderately inhibit ADP-induced aggregation of thrombocytes (see Table 3). A comparison of the

data on the total biological effect over the concentration range 10^{-3} to 10^{-12} M (see Fig. 1) makes it possible to conclude that an increase in the cataphoretic mobility of the thrombocytes by X-XII and XV adequately increases their resistance to the effect of ADP.

The results constitute evidence for the promising character of the search for antiaggregants in series of hydroxyalkylammonium salts of phenylmercaptoacetic acid derivatives.

LITERATURE CITED

1. E. E. Luscher, Helv. Med. Acta, 50, 5 (1971).
2. G. De Gaetano, J. Vermyler, and M. Veratracte, Nouv. Rev. Franc. Hemat., 11, 339 (1971).
3. K. M. Lakin, in: Current Problems in Hemostasiology [in Russian], Moscow (1979), p. 261.
4. M. D. Mashkovskii, Medicinals [in Russian], 77th edn., Vol. 2, Moscow (1972), p. 137.
5. J. McGiff, Triangle, 20, 27 (1980).
6. W. Siess, P. Roth, and P. Weber, Thrombos., Haemostas., 45, 204 (1981).
7. P. Levine, D. Sladdin, and N. Kirnsky, ibid., p. 290.
8. P. Emmons, M. Harrison, et al., Lancet, 2, 603 (1965).
9. K. Rusanek, C. Svehla, H. Spankova, et al., Activ. Nerv. Sup. (Praha), 9, 448 (1967).
10. F. Fabbria, A. Casonato, M. Randi, et al., Folia Haemat. (Leipzig), 108, 433 (1981).
11. R. MacKenzie and T. Blohm, Trombos. Diathes. Haemorrh. (Stuttgart), 26, 577 (1971).
12. A. N. Zhuravlev and Yu. A. Filippov, Zh. Obshch. Biol., 28, 441 (1967).
13. D. J. Pasto and P. Kent, J. Org. Chem., 30, 2684 (1965).
14. S. S. Kharamonenko, in: Problems in the Biophysics, Biochemistry, and Pathology of Erythrocytes [in Russian], Vol. 1, Krasnoyarsk (1960), p. 447.
15. S. A. Rudchenko, Fiziol. Zh. SSSR, No. 6, 771 (1978).

ACETYLENIC AMINOALCOHOLS.

VIII. 3,3-DIPHENYL-3-HYDROXYPROPYNYLPIPERIDINES*

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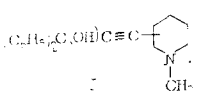
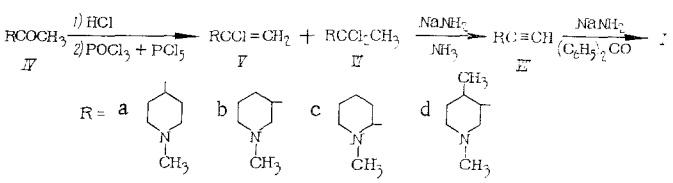
Continuing our search for acetylenic aminoalcohols with cholinolytic properties, we have synthesized some piperidines with the 3,3-diphenyl-3-hydroxypropynyl group in various positions in the ring (I), together with their hydrochlorides (II) and methiodides (III).



These compounds would be expected to have high physiological activity, and furthermore their quite rigid structure should enable their spatial structures to be established with greater certainty, and hence the effect of the latter on cholinolytic activity.

The preparation of compounds of interest to us was carried out from the appropriate piperidine ketones, as follows:



*For Communication VII, see [1].

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