2-HYDROXYALKYLAMMONIUM ARYLTHIOACETATES AND THEIR EFFECTS ON THE FUNCTIONAL ACTIVITY OF THROMBOCYTES

G. G. Levkovskaya, S. A. Guseva,
V. B. Kazimirovskaya, L. N. Kholdeeva,
M. G. Voronkov, and A. N. Mirskova

UDC 615.273.53.015.4:616.155.25

The functional state of thrombocytes plays an important part in the formation of structural thrombi in vessels with rapid blood flow [3].

Many of the currently known compounds which inhibit the aggregation of thrombocytes (such as caffeine, theophyllin, and papaverine) reduce the extent of aggregation of blood formative elements, *in vitro*, but they do not display this activity *in vitro* [8]. The Soviet drug parmidine likewise does not display the same activity *in vitro* and *in vivo* [7]. Hypocholesteremic drugs (clofibrate, cetamifen, et al.), in addition to reducing the blood cholesterol levels, decrease fibrinogen concentration and the tendency to thrombosis, and enhance the effects of anticoagulants [4, 5].

Some amines, such as triethanolamine, diethanolamine, and monoethanolamine also possess antiaggregation activity. Under optimum conditions, triethanolamine derivatives have a membrane-stabilizing effect on the cell structures, the primary mode of action being prevention of the development of free-radical oxidation of the membrane lipids [1].

Tris-(2-hydroxyethyl)ammonium salts of binuclear derivatives of phenylthioacetic acid, previously synthesized by the authors [6], which are similar in structure to the above hypocholesteremic drugs [4, 5] and contain 2-hydroxyalkyl groups, which confer activity on a number of thrombocyte antiaggregants and phospholipids, modify the functional activity of thrombocytes.

In a further search for antiaggregants in the organylthioalkanecarboxylic acids and their derivatives, and a study of structure-activity relationships, we have prepared some 2-hydroxyalkylammonium salts from substituted arylthioacetic acids and 2-hydroxyalkylamines of diverse

TABLE 1. (2-Hydroxyalkyl)ammonium Phenylthioacetates I-XXI

Compound	Yield, %	mp, °C	Found, %			Empirical formula	Calculated, %		
			Cl	N	S		Cl	N	S
I	90	100—102	13,56	5,31	12,24	$C_{10}H_{14}ClNO_3S$	13,45	5,31	12,16
II	82	85,5—87	10,41	4,66	11,26	$C_{12}H_{18}ClNO_4S$	10,52	4,55	11,52
III	83	90—92	10,09	4,00	9,21	$C_{14}H_{22}ClNO_5S$	10,07	3,98	9,11
IV	87	71—73	12,07	4,64	10,40	$C_{12}H_{18}ClNO_3S$	12,15	4,80	10,39
V	89	134—135	10,30	4,56	9,90	$C_{12}H_{17}ClNO_3S$	10,95	4,33	9,90
VI	64	134—138	8,12	4,07	7,97	$C_{18}H_{26}ClNO_5S$	8,78	4,77	7,94
VII	50	131—134	8,60	3,37	7,81	$C_{18}H_{25}ClNO_5S$	8,52	3,36	7,71
VIII	70	83—85	9,20	3,80	8,15	$C_{18}H_{26}ClNO_4S$	9,14	3,61	8,26
IX	50	57—62	10,98	4,62	10,04	$C_{13}H_{17}ClNO_4S$	11,08	4,38	10,03
X	84	—	18,31	3,73	8,20	$C_{14}H_{21}Cl_2NO_5S$	18,39	3,71	8,32
XI	83	123—124	18,32	3,72	8,18	$C_{14}H_{21}Cl_2NO_5S$	18,39	3,71	8,32
XII	80	68—70	—	5,66	12,96	$C_{11}H_{17}NO_3S$	—	5,76	13,18
XIII	60	67,5—69	—	4,70	11,75	$C_{13}H_{21}NO_4S$	—	4,87	11,15
XIV	85	77—77,5	—	4,46	9,70	$C_{15}H_{25}NO_5S$	—	4,22	8,92
XV	96	147—148	—	10,14	11,68	$C_{10}H_{14}N_2O_6S$	—	10,21	11,88
XVI	51	113—114	—	8,71	9,73	$C_{12}H_{18}N_2O_6S$	—	8,79	10,00
XVII	95	107—108	—	7,69	8,70	$C_{14}H_{22}N_2O_7S$	—	7,72	8,84
XVIII	71	147—150	—	11,39	9,12	$C_{10}H_{13}N_3O_7S$	—	11,62	8,33
XIX	89	151—154	—	12,66	9,72	$C_{12}H_{17}N_3O_8S$	—	13,22	10,04
XX	92	144—146	—	11,90	6,96	$C_{17}H_{18}N_4O_{10}S$	—	11,91	6,82
XXI	88	112—118	—	10,29	9,01	$C_{14}H_{21}N_3O_9S$	—	10,31	8,36

Irkutsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 20, No. 3, pp. 295-300, March, 1986. Original article submitted January 29, 1985.

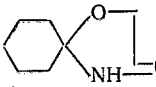
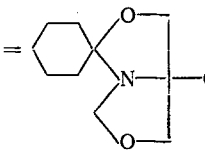
 0091-150X/86/2003-0180$12.50

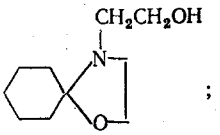
structure, including heterocyclic compounds. Reaction of equimolar amounts of the arylthioacetic acids with the appropriate amines in alcoholic solution at 70-80°C gave the previously unknown 2-hydroxyalkylammonium arylthioacetates

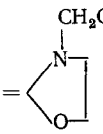
$$RSCH_2COOH + NR^1R^2R^3 \longrightarrow RSCH_2COONHR^1R^2R^3$$

I — XXI

I: R = 4-ClC$_6$H$_4$; $R^1 = R^2 = H$; $R^3 = CH_2CH_2OH$; II: R = 4-ClC$_6$H$_4$; $R^1 = H$; $R^2 = R^3 = CH_2CH_2OH$; III: R = 4-ClC$_6$H$_4$; $R^1 = R^2 = R^3 = CH_2CH_2OH$; IV: R = 4-ClC$_6H_4$; $R^1 = R^2 = CH_3$; $R^3 = CH_2CH_2OH$; V: R = 4-ClC$_6$H$_4$; $R^1 = R^2 = CH_3$; $R^3 = C(CH_2OH)_3$;

VI: R = 4-ClC$_6$H$_4$; $NR^1R^2R^3 =$  $-(CH_2OH)_2$; VII: R = 4-ClC$_6$H$_4$; $NR^1R^2R^3 =$  $-CH_2OH$;

VIII: R = 4-ClC$_6$H$_4$; $NR^1R^2R^3 =$  ;

IX: R = 4-ClC$_6$H$_4$; $NR^1R^2R^3 =$  ; X: R = 2,4-Cl$_2$C$_6$H$_3$; $R^1 = R^2 = R^3 =$ $= CH_2CH_2OH$; XI: R = 2,5-Cl$_2$C$_6$H$_3$; $R^1 = R^2 = R^3 = CH_2CH_2OH$; XII: R = 4-tolyl; $R^1 = R^2 = H$; $R^3 = CH_2CH_2OH$; XIII: R = 4-tolyl; $R^1 = H$; $R^2 = R^3 = CH_2CH_2OH$; XIV: R = 4-tolyl; $R^1 = R^2 = R^3 = CH_2CH_2OH$; XV: R = 4-NO$_2C_6H_4$; $R^1 = R^2 = H$; $R^3 = CH_2CH_2OH$; XVI: R = 4-NO$_2$C$_6$H$_4$; $R^1 = H$; $R^2 = R^3 = CH_2CH_2OH$; XVII: R = = 4-NO$_2$C$_6$H$_4$; $R^1 = R^2 = R^3 = CH_2CH_2OH$; XVIII: R = 2,4-(NO$_2$)$_2C_6H_2$; $R^1 = R^2 = H$; $R^3 = CH_2CH_2OH$; XIX: R = 2,4-(NO$_2$)$_2$C$_6$H$_3$; $R^1 = H$; $R^2 = R^3 = CH_2CH_2OH$; XX: R = = 2,4-(NO$_2$)$_2$C$_6$H$_3$; $R^1 = R^2 = H$; $R^3 = CH(CH_2OH)CH(OH)C_6H_4NO_2$ (d, l- form); XXI: R = 2,4-(NO$_2$)$_2$C$_6$H$_3$; $R^1 = R^2 = R^3 = CH_2CH_2OH$.

The yields, melting points, and elemental analyses of the salts I-XXI are given in Table 1.

It is noteworthy that oxazolidines, which are prone to cleavage to azomethines in the presence of acid, are stable under the reaction conditions, giving the salts VI-IX in high yield.

The IR spectra of VI-IX show absorption at 1620-1630 cm^{-1}, but no band is present which is shifted to higher frequencies, such as would be characteristic of the iminium group $C=\overset{+}{N}<$.

The salts I-XIV were obtained as colorless (compounds XV-XII were yellow) crystalline, odorless solids which were soluble in water, alcohol, and physiological solutions, and were stable on storage with exclusion of moisture.

The IR spectra of I-XXI showed absorption at 1600-1620 cm^{-1} (COO^-) and 3370-3430 cm^{-1} (OH).

EXPERIMENTAL (CHEMICAL)

IR spectra were obtained on a UR-2 spectrometer (KBr disks).

The arylthioacetic acid starting materials were prepared from the appropriate thiols and chloroacetic acid, as described in [12].

4-Nitrophenylthioacetic Acid. 4-Chloronitrobenzene (15.7 g;, 9.2 g of thioglycolic acid, and 10 g of sodium hydroxide in 100 ml of water were boiled for 8 h, cooled, and the solid which separated was filtered off, dissolve in hot water, and acidified with HCl. The crystals which separated were filtered off to give 18.8 g (90%) of 4-nitrophenylthioacetic acid.

Similarly, from 20.3 g of 2,4-dinitrochlorobenzene, 6.9 g of thioglycolic acid, and 9.6 g of NaOH in 100 ml of ethanol was obtained 18.13 g (70%) of 2,4-dinitrophenylthioacetic acid.

5-Hydroxymethyl-1-aza-3,7-dioxabicyclo[3.3.0]octane. Paraformaldehyde (30 g) and 60.5 g

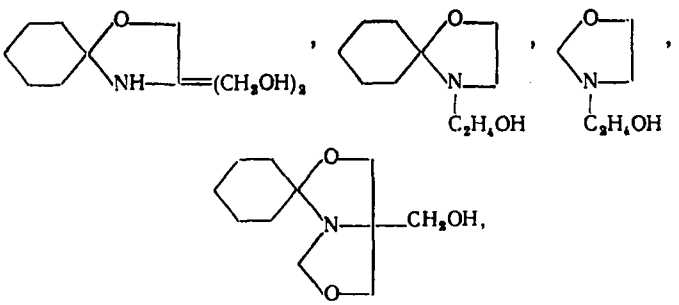
TABLE 2. Maximally Effective Dose and the Overall Value of the Biological Activity (Σ) of (2-Hydroxyalkyl)ammonium Salts of Substituted Phenylthioacetic Acids I-XXI

Compound	Electrokinetic potential			
	effective dose, M	Σ-effect	effective dose, M	Σ-effect
I	10^{-5}—10^{-6}	41	10^{-5}—10^{-7}	93
II	10^{-5}—10^{-7}	45	10^{-5}—10^{-7}	86,8
III	10^{-5}—10^{-6}	60	10^{-5}—10^{-7}	98
IV	10^{-4}—10^{-5}	27	10^{-4}—10^{-5}	61
V	10^{-3}—10^{-5}	20	10^{-3}—10^{-5}	34
VI	10^{-4}—10^{-5}	18	10^{-4}—10^{-5}	53
VII	10^{-3}—10^{-4}	14	10^{-3}—10^{-5}	31
VIII	10^{-3}—10^{-4}	10,5	10^{-3}—10^{-4}	39,5
IX	10^{-3}—10^{-4}	23	10^{-3}—10^{-4}	34
X	10^{-4}—10^{-5}	51,5	10^{-5}—10^{-6}	122
XI	10^{-6}—10^{-7}	50	10^{-5}—10^{-6}	118
XII	10^{-6}—10^{-7}	9	10^{-6}—10^{-7}	20
XIII	10^{-5}—10^{-6}	15,5	10^{-4}—10^{-5}	45
XIV	10^{-4}—10^{-5}	60	10^{-6}—10^{-7}	97
XV	10^{-5}—10^{-6}	35,5	10^{-5}—10^{-6}	62,5
XVI	10^{-3}—10^{-5}	8,5	10^{-4}—10^{-5}	46
XVII	10^{-5}—10^{-6}	40,5	10^{-5}—10^{-6}	98,5
XVIII	10^{-6}—10^{-8}	52	10^{-6}—10^{-8}	99
XIX	10^{-3}—10^{-5}	29,5	10^{-5}—10^{-7}	48,5
XX	10^{-4}—10^{-5}	24,5	10^{-5}—10^{-7}	39,5
XXI	10^{-3}—10^{-4}	12,5	10^{-3}—10^{-4}	41

Note. The Σ-effect is defined as the area (mm^2) under the dose—effect plot over the concentration range 10^{-3}—10^{-12} M.

of tris(hydroxymethyl)aminomethane in 200 ml of benzene were boiled in a flask fitted with a Dean and Stark apparatus until no more water was liberated. After the benzene had been distilled off, the reaction mixture crystallized. Recrystallization from ether gave 58 g (80%) of the oxazolidine.

Similarly, using the method described in [9, 13], the following oxazoldines were obtained, and purified by vacuum distillation:



The purity of the products was checked by GLC, and comparison of the constants with the literature values [9, 13].

Tris-(2-hydroxyethyl)ammonium 2,4-Dinitrophenylthioacetate (XXI). To a solution of 1.24 g of dinitrophenylthioacetic acid in 10 ml of absolute alcohol was added 0.75 g of freshly-distilled triethanolamine in 7 ml of alcohol. The mixture was heated to the boil (75—80°C), then cooled to −5°C. The solid which separated was filtered off and dried to give 1.62 g (83%) of the salt. Compounds I-XX were obtained similarly.

EXPERIMENTAL (BIOLOGY)

The potential thrombocyte aggregation inhibitors were screened by measuring the electrokinetic potential of the blood cells [10] over the concentration range 10^{-3}—10^{-12} M.

Normal thrombocyte-rich citrated plasma was incubated at 37°C for 15 min in the presence of the test compound. Before and after incubation, the decrease in optical density (DOD) was measured as a percentage of the control. Following incubation, ADP was added to the samples in a concentration of 200 μg/ml.

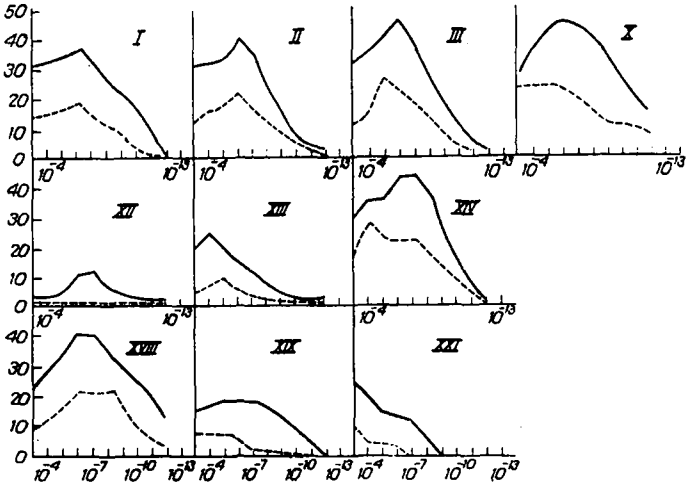


Fig. 1. Changes in the functional activity of thrombocytes in the presence of (2-hydroxyethyl) ammonium salts of substituted phenylthioacetic acids. The broken line represents the changes in electrophoretic mobility of the thrombocytes as a percentage of that of untreated thrombocytes (0.76 + 0.04 $mm \cdot sec^{-1} \cdot cm^{-1}$), and the solid line the inhibition of aggregation as a percentage of the controls (ADP final concentration 200 μ/mg). The horizontal axis represents concentration (M), and the vertical axis these values (%). Compounds tested: I, II, III, X, XII, XIII, XIV, XVIII, XIX, XXI.

Surface charge was measured from the rate of movement of the cells in an electric field, and the increase in charge relative to the control calculated (as a percentage).

Tests were carried out with plasma of the blood of rabbits, obtained from the marginal vein of the ear [11]. The blood, stabilized in a ratio of 9:1 with sodium citrate solution, was centrifuged for 10 min at 1000 rpm.

The changes in functional activity of the thrombocytes in the presence of salts I-XXI are shown in Table 2 and Fig. 1.

It will be seen from Fig. 1 that treatment with the compounds results in inhibition of thrombocyte aggregation, accompanied by corresponding changes in their electrokinetic potential. The inhibitory effect, both with respect to thrombocyte aggregation and changes in electrophoretic charge, is dependent on the substituents in the benzene ring, the numbers thereof, and the structure of the ammonium cation. The data presented in Table 2 show that of the 4-chlorophenylthioacetic acid derivatives, the mono-, bis-, and tris-(2-hydroxyethyl)ammonium salts show the greatest antiaggregation activity as compared with the derivatives of other alkanolamines. For example, the 4-chlorophenylthioacetate salts (I-III), which contain the mono-, bis-, and tris-(2-hydroxyethyl)ammonium cations, are most active at a concentration of 10^{-6} M, inhibition of thrombocyte aggregation at this dose being 36, 40, and 45% respectively. Sodium 4-chlorophenylthioacetate gives 25% inhibition at the higher concentration of 10^{3} M. The presence of the hydroxyethyl radicals in salts I-III in all likelihood is responsible for the increased affinity of these compounds for the thrombocyte cell membranes. Mono-, di-, and triethanolamine hydrochlorides possess antiaggregation activity, but to a much lower extent, and at higher concentrations than (I-III). The increased inhibition of aggregation in the latter could be due to synergism. A study of a series of 4-tolylthioacetic acid derivatives showed that increasing the numbers of 2-hydroxyethyl groups in the ammonium moiety in salts XII-XIV also resulted in increased antiaggregation activity. As compared with the corresponding 4-chlorophenylthioacetates I-III and 4-nitrophenylthioacetates XV-XVII, the dependence of antiaggregation activity on the number of 2-hydroxyethyl groups in the ammonium fragment was more complex. In all the cases mentioned, however, the maximum inhibitory effect was obtained with the tris-(2-hydroxyethyl)ammonium salts. In the case of the series mono-, bis-, and tris-(2-hydroxyethyl)ammonium 2,4-dinitrophenylthioacetates (XVIII, XIX, and XXI), however, the inverse relationship was found, the antiaggregation activity decreasing with the number of

2-hydroxyethyl groups.

Incorporation of cations containing the oxazolidinium ring (VI-IX) reduces the biological activity by a factor of approximately 2-3 as compared with the mono-, di-, and triethanolamine compounds.

Maximum inhibitory activity in the tris-(2-hydroxyethyl)ammonium salts of substituted arylthioacetic acids is found in compounds containing one or two chlorine atoms (III, X, XI) or a nitro-group (XVII) as electron-acceptor substituents. The derivatives of the methyl-substituted acids (XII and XIII) are much less active, both in respect of changes in electrophoretic charge, and thrombocyte aggregation, as compared with compounds which contain electron-acceptor substituents in the benzene ring. The triethanolamine derivative XIV has antiaggregation activity comparable with that of tris-(2-hydroxethyl)ammonium 4-chloro-and 4-nitrophenylthioacetates.

When two nitro-groups were introduced into the benzene ring, only in the case of the monoethanolamine salt was the antiaggregation activity not reduced in comparison with that of XVII.

The great majority of the compounds obtained were of low toxicity (3000 mg/kg < LD_{50} < 1340 mg/kg). Exceptions were (V) and (XI), the toxicities of which were considerably greater (LD_{50} 53.3 and 120 mg/kg respectively).

All of the compounds tested therefore possess antiaggregation activity. They display activity which is of the same type but somewhat different in degree on the dynamic functions of thrombocytes.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Peroxide Oxidation of Lipids in Biological Membranes [in Russian], Moscow (1972).
2. A. N. Zhuravlev and Yu. A. Filippov, Zh. Obshch. Biol., 28, 441-448 (1967).
3. F. I. Komarov, in: Antithrombotic Therapy in Clinical Practice [in Russian], Moscow (1982), p. 3.
4. K. M. Lakin, V. A. Fel'dbaum, and A. A. Lebedeva, Farmakol. Toksikol., No. 1, 104-113 (1971).
5. K. M. Lakin, in: Current Problems in Hemostasiology [in Russian], Moscow (1979), p. 261.
6. G. G. Levkovskaya, Yu. I. Kryukova, Yu. A. Moskvichev, et al., Khim.-farm. Zh., No. 4, 431-435 (1984).
7. M. D. Mashkovskii, K. M. Lakin, M. S. Ovnatanova, and G. Ya. Shvarts, Byull. Éksp. Biol., No. 3, 332-334 (1976).
8. V. E. Pasterova, Usp. Fiziol. Nauk, No. 2, 132-152 (1977).
9. V. B. Sokolov, L. N. Kryukov, A. F. Kolomets, and G. A. Sokol'skii, Zh. Org. Khim., No. 8, 1777-1779 (1979).
10. S. S. Kharamonenko, in: Topics in the Biophysics, Biochemistry, and Pathology of Erythrocytes [in Russian], 447 (1960).
11. J. V. R. Born, Nature, 194, 927-929 (1962).
12. D. J. Pasto et al., J. Org. Chem., 30, 2684-2687 (1965).
13. M. Sencus, J. Am. Chem. Soc., 67, 1515-1519 (1945).