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SYNTHESIS AND STUDY OF CERTAIN NEW DISULFIDE DERIVATIVES OF OXYTHIAMINE

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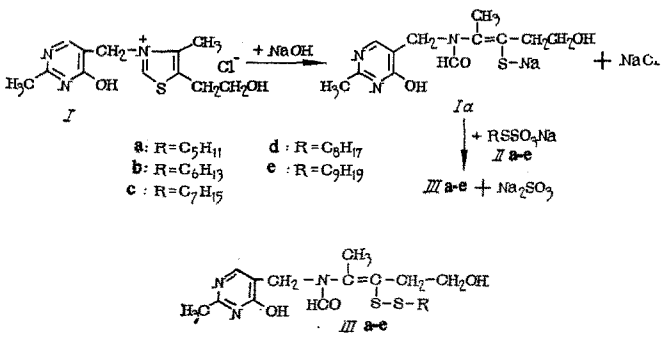
The use of antivitamins as medicinal preparations is one of the modern trends in chemotherapy. But these compounds are usually used in experimental practice as specific agents for the inhibition of different vitamin-dependent reactions [1]. The attention of many authors is at present drawn to thiamine antimetabolites, which are artificially obtained derivatives of vitamin B_1. To produce new effective thiamine antivitamins, taking into consideration that two of its disulfide compounds, which are very valuable vitamin and medicinal preparations, are already known [2-4], we synthesized a series of oxythiamine derivatives, which are 2-methyl-4-hydroxy-5-{N-formyl-N-[1-methyl-2-alkyldithio-2-(2-hydroxyethyl)-ethenyl]-aminomethyl} pyrimidines of the general formula (III):



Sodium alkylthiosulfate (II) was used as the thiolating agent.

To obtain the above compounds by this method, oxythiamine (I) in aqueous solution at pH $\geq$ 10.0, is converted into the highly reactive thiol form (Ia). The end products (IIIa-e) are then synthesized by reaction with the corresponding sodium alkylthiosulfate (IIa-e), obtained from alkyl bromide and sodium thiosulfate in an aqueous-alcoholic solution. The disulfide derivatives of oxythiamine are hygroscopic white amorphous powders, which are soluble in water, aliphatic alcohols, pyridine, and ethyl acetate, but are insoluble in toluene,

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TABLE 1. Disulfide Derivatives of Oxythiamines IIIa-e

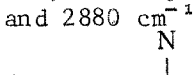
Compound	Yield, %	mp, deg C	Found, %		Empirical formula	Calculated, %	
			N	S		N	S
IIIa	48	105	11,04	16,60	$C_{17}H_{27}N_3O_3S_2$	10,89	16,63
IIIb	63	106	10,32	16,13	$C_{18}H_{29}N_3O_3S_2$	10,51	16,04
IIIc	61	106—107	10,02	15,44	$C_{19}H_{31}N_3O_3S_2$	10,15	15,50
IIId	60	107	9,71	14,85	$C_{20}H_{33}N_3O_3S_2$	9,82	14,99
IIIe	59	109	9,35	14,43	$C_{21}H_{35}N_3O_3S_2$	9,51	14,51

TABLE 2. Activity of Pyruvate Dehydrogenase (in μmole of $K_3[Fe(CN)_6]$ per g of Protein) in Liver of White Mice at Different Periods of Time after Single Administration of Compounds IIIa-e (M ± m)

Compound		LD_{50}, mg/kg	Time interval, h			
			1	3	12	24
Control		—	107,0±7,0	126,0±2,7	149,0±6,0	160,0±8,0
Oxythiamine		1430	79,0±5,0	65,0±10,0	85,0±5,0	138,0±8,0
	P_1		<0,01	<0,05	<0,001	<0,001
IIIa		1250	31,0±8,3	31,3±11,0	37,0±7,0	46,0±10,0
	P_1		<0,001	<0,02	<0,001	<0,001
	P_2		>0,001	<0,05	>0,001	>0,001
IIIc		1045	25,0±6,3	33,0±2,6	41,0±8,0	49,0±4,8
	P_1		<0,001	>0,01	<0,001	<0,001
	P_2		0,001	<0,02	>0,001	>0,001
IIId		780	29,0±4,0	29,0±7,6	51,0±5,8	40,0±3,7
	P_1		<0,001	>0,01	<0,001	<0,01
	P_2		<0,001	>0,02	>0,001	<0,01
IIIe		570	28,0±6,0	26,0±6,3	46,0±6,7	30,0±5,4
	P_1		<0,001	>0,01	<0,001	<0,001
	P_2		0,001	>0,02	<0,001	<0,02

Note. Here and in Table 3: P_1 is reliability of differences with respect to control, P_2 compared with the parameter in animals receiving oxythiamine.

benzene, dioxane, acetone, hexane, heptane, or petroleum ether. The structure of the disulfides synthesized was confirmed by the elemental analysis data, and IR and UV spectroscopically. In the IR spectra of these compounds characteristic bands at 3450 cm^{-1} are present, corresponding to the stretching vibrations of associated hydroxyl groups, in the 2950 and 2880 cm^{-1} region of the =CH—, $-CH_2-$, and $-CH_3$ groups, at 1690 cm^{-1} of the pyrimidine ring



and the —HCO— grouping, at 1590 cm^{-1} of the —C=C— group, and bands in the 1430 and 1390 cm^{-1} region corresponding to the deformational vibrations of the $-CH_2-$ and $-CH_3$ groups.

The UV spectra of compounds IIIa-e are similar, and are characterized by one single absorption maximum at 267 nm.

EXPERIMENTAL CHEMICAL SECTION

The IR spectra of compounds IIIa-e were run on the "UR-20" spectrometer (GDR) in KBr tablets, and the UV spectra were measured on the "Specord-UV-VIS" spectrophotometer (GDR) in phosphate buffer solutions (pH 7.0). The individual state of the disulfide derivatives of oxythiamine obtained was confirmed by ascending chromatography method. Inspection of chromatograms obtained in the butanol—ethanol—water (2:1:1) system of solvents (paper "Filtrak" No. 11, GDR) in UV light revealed one spot with R_f 0.93 given by compounds synthesized. Before their purification with liquid hydrocarbons, up to 3 spots with R_f 0.34, 0.59, 0.93 were found on the chromatograms in this system of solvents. The spot with R_f 0.93 was the most intense. In the experiments with reference spots it was found that the two other spots correspond to oxythiamine (R_f 0.34) and oxythiamine disulfide (R_f 0.59).

Oxythiamine Amyl Disulfide (IIIa). A 5-g portion (0.0148 mole) of oxythiamine is dissolved with stirring and cooling (5°C) in 5 ml of water, a 40% aqueous solution of NaOH is added dropwise to pH > 10.0, and sodium chloride is added to saturation. After 30 min, 4.5 g (0.022 mole) of sodium amylthiosulfate are added in portions, and the reaction mixture is stirred for 3 h at 18-20°C, with continuous control of the pH of the medium, which should be

TABLE 3. Activity of Transketolase (in μmole of Seduheptuloso-7-phosphate per g of Tissue per h) in Liver of White Mice after Single Administration of Compounds IIIa-e (M ± m)

Compound		Time interval, days				
		2	3	5	8	12
Control		210,0±8,0	210,0±8,0	205,0±11,0	205,0±11,0	210,0±9,2
Oxythiamine		128,0±5,2	134,0±12,0	174,0±11,0	181,0±12,8	200,0±8,0
	P_1	<0,001	>0,001	>0,05	>0,2	>0,5
IIIa		99,0±3,4	105,0±7,1	108,0±6,0	132,0±4,7	145,0±5,3
	P_1	<0,001	<0,001	<0,001	<0,001	<0,001
	P_2	>0,01	>0,05	>0,001	>0,001	>0,001
IIIc		128,0±6,9	97,0±4,6	101,0±8,5	122,0±6,0	151,0±7,1
	P_1	<0,001	<0,001	<0,001	<0,001	>0,001
	P_2	—	>0,02	>0,001	<0,01	<0,001
IIId		101,0±6,9	97,0±10,7	94,0±7,9	119,0±7,1	137,0±6,9
	P_1	<0,001	<0,001	<0,001	<0,001	<0,001
	P_2	<0,02	<0,05	<0,001	<0,01	0,001
IIIe		80,0±5,4	90,0±11,8	101,0±5,6	130,0—8,3	141,0—8,7
	P_1	<0,001	<0,001	<0,001	>0,001	>0,001
	P_2	<0,001	<0,001	0,001	>0,01	>0,001

not lower than 10.0. The material is extracted with ethyl acetate (3 × 5 ml), neutralized with an alcoholic solution of HCl to pH 7.0-7.5, and filtered from sodium chloride. The solvent is evaporated *in vacuo* (25°C) and the product dried over P_2O_5. The residue is dissolved in 10 ml of butanol, then separated from impurities by filtration or centrifugation, and the transparent alcoholic solution is poured into 50 ml of heptane. These operations are repeated, and the residue is washed several times with heptane and dried *in vacuo* to yield 2.74 g (48%) of 2-methyl-4-hydroxy-5-{N-formyl-N-[1-methyl-2-amyldithio-2-(2-hydroxyethyl)-ethenyl]aminomethyl}pyrimidine (IIIa).

Compounds IIIb-e were synthesized in a similar way. But, since with increase in the alkyl radical the solubility of the compounds in hydrophobic solvents increases, the purification of compounds IIIb-e is carried out from ethyl acetate solutions. The yields, melting points and data of elemental analysis of the disulfide derivatives obtained are listed in Table 1.

EXPERIMENTAL PHARMACOLOGICAL SECTION

The studies were carried out on male white mice, weighing 16-18 g each, fed on a rich food ration. For 12 h before decapitation, the animals did not receive any food. The disulfide derivatives of oxythiamine were introduced by a single subcutaneous administration in the form of aqueous solutions, in isomolar amounts with respect to oxythiamine, in a dose of 200 mg/kg. The effective toxic doses of the preparations were determined on white mice with subcutaneous administration according to the method described in [5].

The antivitamin activity of compounds IIIa-e were evaluated from the inhibition of the activity of transketolase and dehydrogenases of α-ketoglutaric and pyruvic acids, whose change in activity can serve as an objective criterion in the evaluation of the vitamin status of the organism. The amount of thiamine diphosphate in the liver of the animals was also studied. The activity of transketolase was determined by the method described in [6], pyruvate- and α-ketoglutarate dehydrogenase by the method in [7], and the content of thiamine diphosphate was found by an enzymatic method using apopyruvate decarboxylase [8, 9]. The activity of the chloropyruvate decarboxylase formed is directly proportional to the content of thiamine diphosphate and was determined by the method described in [10]. The data were statistically treated by a conventional method [11].

Our investigations showed (Tables 2 and 3) that disulfide derivatives IIIa-e synthesized have a low toxicity, and surpass oxythiamine in their antimetabolite action. A special advantage of these antivitaminic compounds is their more prolonged action in the organism after a single administration.

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RELATIVE BIOLOGICAL ACTIVITY OF VITAMINS D_2, D_3 AND THEIR 1α-HYDROXY ANALOGS IN THE CHICKEN

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Vitamins D_2 and D_3 are equivalent with respect to their biological activity in mammals, but in poultry, vitamin D_3 is considerably more active than vitamin D_2 [1]. In recent years it was found that this discrimination in poultry is extended also to active metabolites, the 25-hydroxy vitamin D_2 ($25(OH)D_2$) [2] and 1α,25-dihydroxyvitamin D_2 ($1\alpha,25\text{-}(OH)_2D_2$) [3], although in the case of rats the metabolites of the two forms are equivalent [4]. It should, however, be noted that a quantitative evaluation of the ratio of the activities of vitamins D_2 and D_3 varies, and in separate experiments is 10 [5], 40 [6], and 60 [7]. There are very few data on the activity of the metabolites of these vitamins and synthetic analogs, in particular, 1α-hydroxyvitamins D.

The considerable divergences in the evaluation of the activity of vitamins D_2 and D_3 in poultry can to a certain extent be explained by difference in the selection of the biological criterion of their action in the organism used in the experiment. As the result of the latest achievements in the study of the action mechanism of vitamin D in animals, as the most specific biological test we can suggest the synthesis in the intestinal epithelium of a vitamin D dependent calcium binding protein (CaBP) participating in the calcium absorption process [8]. It is also important that there is a direct correlation between the dose of vitamin D and CaBP in the mucous membrane of small intestines, so that this parameter can be used for the quantitative determination of the activity of vitamin D_3 and its analogs in the organism [9].

The aim of the present work was a comparative study of the biological activity in poultry of vitamin D_2 and D_3 preparations and their 1α-hydroxy-analogs. The activity was evaluated with respect to seven parameters, and the action of the preparation on CaBP biosynthesis was used as the most important criterion.

From the data obtained, and taking into account the set of parameters, it was found that vitamin D_3 is 15 times, and $1\alpha(OH)D_3$ 10 times, more active than vitamin D_2 and $1\alpha(OH)D_2$, respectively. Thus, the discrimination with respect to vitamin D_2 becomes weaker in the presence of a hydroxy group at the 1α-position in the molecule.

EXPERIMENTAL

Two experiments were carried out on young white Leghorn roosters: one with a long-term administration of the preparation, and the other, a short-term experiment with a single administration of a 72-h ration.

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