SYNTHESIS, STRUCTURE, AND ACTION OF SOME GOSSYPOL DERIVATIVES ON THE PEROXIDATION OF THE LIPIDS OF BIOSUBSTRATES

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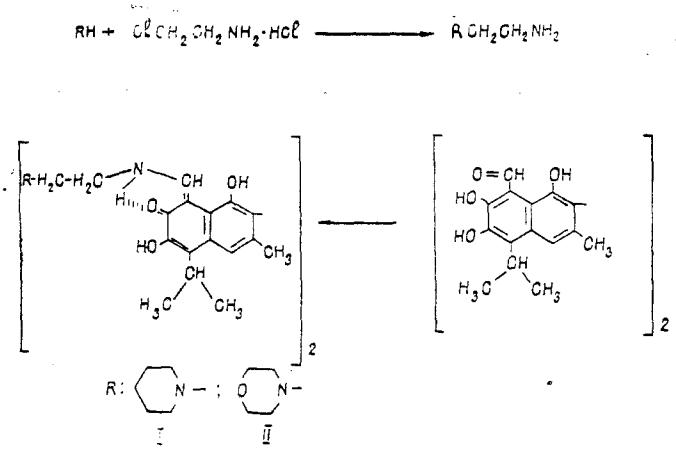
The synthesis has been effected of new gossypol derivatives using piperidino- and morpholinoethylamines. According to their PMR spectra, these substances exist in the keto-amine form. Their action on the peroxidation of lipids (POL) of various biosubstrates has been studied. Gossypol bis(piperinoethylimine) and bis(morpholinoethylimine) in concentrations of $1 \cdot 10^{-7}$-$5 \cdot 10^{-6}$ mM exert a pronounced antioxidant action on human blood serum and rat brain synaptosomes. In the same concentrations, these substances suppressed the POL in enzymatic and nonenzymatic systems of the oxidation of rat liver microsomes.

Gossypol, obtained from natural raw material, is an antiradical inhibitor of the phenol type. The mechanism of its action consists in giving up labile hydrogen to free radicals with the formation of radicals having a low activity and the termination of the lipid peroxidation chains [1]. Gossypol derivatives have recommended themselves as effective inhibitors of free-radical processes; their antiradical activity (ARA), studied by the chemiluminescent method (CL) on an ethylbenzene–acetic acid model, considerably exceeded the ARA of Ionol (2,6-di-tert-butyl-4-methylphenol) [2].

We have synthesized new gossypol derivatives and tested their antioxidant action on various biosubstrates: the blood sera of healthy donors, rat brain synaptosomes, and rat liver microsomes.

The gossypol derivatives were synthesized by the following scheme:



As substituents we selected piperidinoethylamine and morpholinoethylamine, since derivatives of these heterocycles are highly specific inhibitors (substrates) of acetylcholinesterase [3]. On the other hand, the introduction of electron-donating substituents into a binaphthyl system leads to an increase in antioxidant activity [2].

In the PMR spectrum of gossypol bis(piperidinoethylimine) there were signals characteristic for the gossypol itself: A doublet of the methyl groups of an isopropyl fragment was located in 1.44 ppm; a singlet at 2.05 ppm corresponded to an aromatic methyl group; a multi-

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plet at 3.56 ppm belonged to the methine proton of an isopropyl radical; and, finally, a singlet at 7.42 pm related to the H_4 aromatic proton. The presence of a doublet at 9.48 ppm and of a broad singlet at 14.2 ppm belonging to exomethine and NH protons, respectively, indicated the existence of the molecule of (I) in the keto-amine form in solution [4]. When traces of D_2O were added or the NH proton was irradiated with a second powerful high-frequency field (total resonance), the signal of the exomethine proton changed into a singlet. The signals of the other protons of the substituent were present in the following positions: a multiplet at 3.45 ppm belonged to the methylene protons of a $-CH_2-NH-$ group. The splitting of this signal was due to spin–spin coupling with the neighboring methylene proteins and the NH proton, the rate of hydrogen exchange of which was lowered because of its participation in a strong intramolecular hydrogen bond. A triplet of 2.37 ppm was due to the resonance of the $N-CH_2$ protons of the piperidine ring. The other protons of the piperidine ring formed a structureless signal in the 1.2-1.6 ppm region.

The parameters of the PMR spectrum of gossypol bis(morpholinoethylimine) also showed that it was present in the keto-amine form.

The IR spectra of substances (I) and (II) have peaks corresponding to the vibrations of an OH group of the gossypol fragment at 3560 cm^{-1} and of a C=N bond at 1685 and 1640 cm^{-1}; consequently, these substances were undergoing keto-imine tautomerism, passing from the keto form into the imine form, as was also confirmed by the PMR spectra.

The UV spectrum of substance (I) had an absorption peak at $\lambda_{max}^{CH_3COCH_3}$ 400 nm, and that of substance II at $\lambda_{max}^{CH_3COCH_3}$ of 350 nm. Since substances (II) contained an electron-accepting morpholine heterocycle the $n-\pi^*$ transition observed took place at shorter wavelengths [6].

The action of these compounds on the level of the POL (peroxidation of lipids) of various biosubstrates was investigated. The level of POL in the lipids of blood serum and synaptosomes was studied by the method of Fe-induced CL. The rate of oxidation of the lipids in the presence of the compounds was evaluated from the change in the angle of slope of the slow flash [6, 7].

It can be seen from Fig. 1a, b that compounds (I) and (II) caused a fall in the level of POL even at a concentration of $3 \cdot 10^{-7}$ M in the lipids both of serum and of synaptosomes. They showed a more pronounced antioxidant action on blood serum, which was due to the high oxidizability of the serum lipoproteins [8].

So far as concerns the antioxidant action on rat brain synaptosomes (Fig. 1a), at a concentration of these substances in the sample of up to $6 \cdot 10^{-7}$ M the fall in the level of POL in the synaptosomes was the same. However, at a concentration of $1 \cdot 10^{-5}$ M, substance (I) lowered the POL by 68%, and substance (II) did so by 45%.

As standard we took a well-studied antioxidant – Ionol [9]. Up to a concentration of $1 \cdot 10^{-6}$ M, Ionol lowered the level of POL less effectively than substances (I) and (II); a clearly defined level of lowering of the POL in the presence of Ionol was observed in a narrow concentration range of from $1 \cdot 10^{-6}$ to $3 \cdot 10^{-6}$ M, and with a further increase in its concentration no oxidative processes were recorded (see Fig. 1a). In concentrations of up to $1.6 \cdot 10^{-6}$ M, substances (I) and (II) suppressed the intensity of CL more strongly than Ionol, which shows their great antioxidant (AO) effect on synaptosome membranes in low concentrations.

On blood serum, the AO actions of substances (I) and (II) differed considerably (Fig. 1b). In a concentration of $6 \cdot 10^{-7}$ M, gossypol bis(piperidinoethylimine) lowered the level of CL by 62.8%, while gossypol bis(morpholinoethylamino) did so by 13%. The action of Ionol in a concentration of $3 \cdot 10^{-7}$ M on blood serum lowered the level of CL by 69.1%. It can be seen from the results presented that the AO action of gossypol derivatives depends not only on the electron-donating properties of the substituents but also on the oxidizability of the biosubstrates studied. In blood serum, the antioxidant activity (AOA) of gossypol bis(piperidinoethylamine) was shown more powerfully than in synaptosomes, which was due to the ready oxidizability of the blood serum lipids. The influence of these substances on the POL of rat liver microsomes was studied. It can be seen from Fig. 1c, d, that substances (I) and (II) lowered the yield of malondialdehyde (MDA) both in ascorbate- and in NADPH-dependent oxidation systems. A 50% inhibition of the yield of MDA in comparison with control (without an inhibitor) for substance (I) in NADPH- and ascorbate-dependent systems took place at concentrations of $3.2 \cdot 10^{-6}$ and $1.4 \cdot 10^{-6}$ M, and for substance (II) at $8 \cdot 10^{-6}$ and $5 \cdot 10^{-6}$ M, i.e.,

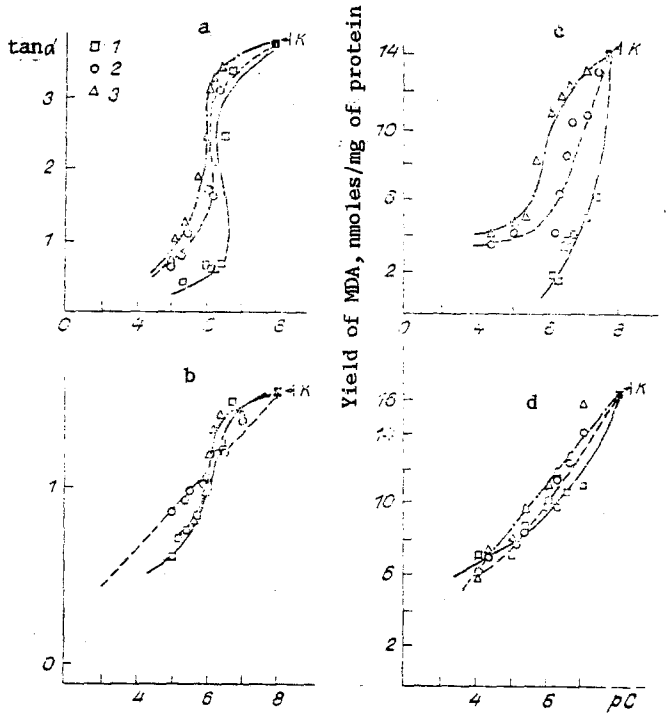

Fig. 1. Interaction of Ionol (1), gossypol bis(piperidinoethylimine) (2), and gossypol bis(morpholinoethylimine) (3) with rat brain synaptosomes (a) and with healthy donor blood serum (b). Ascorbate-dependent (c) and NADPH-dependent (d) oxidation of rat liver microsomes (pC – negative logarithm of the molar concentration of the substances).

their inhibiting effects in two oxidation systems were approximately the same. Substance (I) lowered the yield of MDA more powerfully than (II). Consequently, the action of these substances on the POL biosubstrates and on the yield of MDA in microsomes were in the same direction and obeyed the same law. For Ionol, a 50% suppression of the yield of MDA in comparison with control took place in NADPH- and in ascorbate-dependent systems in concentrations of $8 \cdot 10^{-7}$ and $4.2 \cdot 10^{-8}$ M; consequently, Ionol inhibited the POL of microsomes in an ascorbate system more strongly. Thus, the new gossypol derivatives synthesized have proved to be effective antioxidants. In action on rat brain synaptosomes in low concentrations they possessed more pronounced antioxidant properties than Ionol. These substances as membrane-addressed antioxidants may be effective modulators of oxidative processes in neuronal structures.

EXPERIMENTAL

The IR spectra of the solutions of the substances in carbon tetrachloride were taken on a Specord 71-IR spectrophotometer in NaCl cells over the interval of absorption of the spectra of 4600-700 cm^{-1}. PMR spectra were recorded on a XL-200 spectrometer (Varian) with a working frequency of 200 MHz. The samples used were 5% solutions of the substance under investigation in CCl_4 and $CDCl_3$. Chemical shifts were measured in the δ scale relative to the internal standard HMDS. UV spectra were taken on a SF-26 spectrophotometer using 0.002% solutions in acetone.

Synthesis of Piperidinoethylamine. At 20°C, with stirring, 10 g (0.086 mole) of β-chloroethylamine hydrochloride [16] was added over 30 min to 33.3 ml of a 30% (0.116 M) aqueous solution of piperidine, and the mixture was heated at 35°C for 15 min and at 62-65°C for 8 h. Then it was cooled to 20-30°C and 18.4 g of NaOH (0.46 mmole) was added over 30 min, and the reaction mixture was stirred for another 30 min and was left overnight at room temperature. The upper layer that had formed was separated off and was dried over alkali and redistilled (morpholinoethylamine was obtained similarly).

The boiling point of the piperidinoethylamine was 125-127°C; yield 6.6 g (44.6% of theory), d_4^{20} 0.8933, n_D^{20} 1.4663, MR_{Dfound} 39.7, MR_{Dcalc} 39.69.

The boiling point of the morpholinoethylamine was 150-152°C, yield 5.9 g (40.3% of theory), d_4^{20} 0.9800, n_D^{20} 1.4654, MR_{Dfound} 36.9, MR_{Dcalc} 36.71.

Synthesis of Gossypol Bis(piperidinoethylimine). With heating on the water bath for 2 h, 0.91 g (0.0017 mole) of gossypol was dissolved in 14 ml of ethanol, and this solution was treated with 0.5 g (0.034 mole) of piperidinoethylamine dissolved in 10 ml of ethyl acohol. The mixture was heated to the boiling point of the solvent for 3 h and was left overnight at room temperature. The dark-colored precipitate that had deposited was filtered off and it was washed first with alcohol and then with ether to eliminate unchanged gossypol. It was dried in a vacuum drying chest at 50-60°C, mp 181-182°C (decomp.), yield 0.71 g (55% of theory), Gossypol bis(morpholinoethylamine) was obtained similarly. mp 178-180°C (decomp.), yield 0.52 g (40% of theory), N_{found} 7.5%; N_{calc} 7.58%.

The apparatus for measuring chemiluminescence consisted of a FÉU-39A low-noise photoelectron multiplier and an electrometric amplification system recording on the chart of a KSP-4 recording potentiometer.

The microsomal fraction from rat liver was obtained as described in [10]. The POL activity in the experiments with the NADPH- and ascorbate-dependent oxidation systems was studied from the yield of malondialdehyde determined from the reaction with thiobarbituric acid [11].

The synaptosomes were obtained as in [12]. Their activity was determined by Ellman's method [13]. Protein concentrations in the biosubstrates were determined by Bradford's method [14].

Fe-induced chemiluminescence was produced in accordance with [6]. At room temperature, 0.5 ml of blood serum (3 mg of lipids, 3-4 mg of protein) was added to 8 ml of 105 mM KCl, 20 mM phosphate buffer, pH 7.4, and then 0.5 ml of 10 mM $KMnO_4$ was used. The sample was transferred into the stirrer-containing thermostated cell of the chemiluminometer, the shutter was opened, the recorder was switched on, and level of spontaneous CL was recorded, after which 1 ml of a freshly prepared 10 mM solution of $FeSO_4$ was added and the CL parameters were recorded for 10 min. On the recorder chart the tangent to the curve describing the slow flash was drawn and the angle formed by the tangent to the X axis was determined and its tangent was taken.

The CLs of the substances being studied were recorded in triplicate. The POL with respect to the yield of MDA in the microsomal fractions was determined from six measurements. The chemiluminescence of rat brain synaptosomes was determined in a similar way to the CL of the blood serum.

SUMMARY

1. Gossypol bis(piperidinoethylimine) and bis(morpholinoethylimine), which exist in solution in the keto-amine form, have been synthesized.

2. In concentrations of $1 \cdot 10^{-7}$-$5 \cdot 10^{-6}$ M, the compounds obtained exerted a pronounced antioxidant action on human blood serum and rat brain synaptosomes. In low concentrations these substances suppressed CL in rat brain synaptosomes more powerfully than Ionol. In the same concentrations, these substances suppressed POL in enzyme- (NADPH-) and nonenzyme-(ascorbate-)dependent systems of the oxidation of rat brain microsomes.

LITERATURE CITED

1. A. K. Karabanovich, N. I. Baram, and A. I. Ismailov, in: Abstracts of Lectures at the 4th All-Union Symposium on Phenolic Compounds [in Russian], Tashkent (1982), p. 23.
2. N. A. Zakharova, G. N. Bogdanov, K. E. Kruglyakova, N. M. Emanuel', A. S. Sadykov, A. I. Ismailov, L. Biktimirov, and N. I. Iskhakov, The Chemistry of Plant Substances [in Russian], TashGU, Tashkent, Vol. 3 (1968), p. 90.
3. A. A. Abduvakhabov, A. A. Sadykov, D. N. Dalimov, and Kh. A. Aslanov, Alkaloids and Their Derivatives as Tools for Studying Cholinergic Systems [in Russian], Tashkent (1984), p. 288.
4. L. Biktimirov, N. I. Baram, F. G. Kamaev, A. I. Ismailov, and V. B. Leont'ev, Khim. Prir. Soedin., No. 2, 286 (1975).
5. N. M. Émanuel', Experimental Methods of Chemical Kinetics [in Russian], Vysshaya Shkola (1971), p. 174.

6. Yu. A. Vladimirov and A. I. Archakov, The Peroxide Oxidation of Lipids in Biological Membranes [in Russian], Nauka, Moscow (1972), p 252.
7. Yu. M. Lopukhin, Yu. A. Vladimirov, M. N. Molodenkov, et al., Byull. Éksp. Biol. Med., 2, 61 (1983).
8. R. Harrison and G. G. Hunt, Biological Membranes, Blackie, Glasgow (1975), p. 153.
9. E. B. Burlakova, A. V. Alekseenko, E. N. Molochkina, N. P. Pal'mina, and N. G. Khrapova, Bioantioxidants in Radiation Diseases and Malignant Growth [in Russian], Nauka, Moscow (1975), p. 214.
10. A. V. Archakov and N. M. Devichevskii, Biokhimiya, 33, 479 (1968).
11. V. A. Kostyuk and E. F. Lunets, Biokhimiya, 48, No. 9, 1491 (1983).
12. E. L. Gray and V. P. Whittaker, J. Anat., 96, No. 1, 79 (1962).
13. G. L. Ellman, K. D. Courtney, V. Anders, and R. M. Featherstone, Biochem. Pharmacol., 7, 88 (1961).
14. M. M. Bradford, Anal. Biochem., 72, No. 12, 248 (1976).
15. Yu. V. Markova, K. K. Kuz'mina, M. N. Shchukina, Zh. Obshch. Khim., 30, 1039 (1950).

TRANSFORMATIONS OF GOSSYPOL IN LOW-MOLECULAR-MASS ALCOHOLS

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It has been shown that the transformations of gossypol into anhydro derivatives on its storage in methanol, ethanol, and propanol take place the faster the lower the molecular mass of the alcohol and the higher the temperature of the process. It has been established that when alcoholic solutions of gossypol in methanol are heated it is converted completely into dianhydrogossypol, in propanol into monoanhydrogossypol, and in ethanol into a mixture of equal amounts of mono- and dianhydro derivatives.

We have established previously that, in methanol, ethanol, and propanol, gossypol undergoes degradation leading to the formation of mono- and dianhydrogossypols [1]. In the present paper we consider the influence of the molecular masses of the alcohols, the temperature, and the time of storage on the rate of transformation of gossypol. The rate of change was judged from the decrease in the concentration of aldehyde groups in the products obtained.

To determine the amount of aldehyde groups we used the capacity of alcoholic solutions of gossypol for absorbing light intensively in the UV region of the spectrum. The absorption maximum at 376-378 nm in ethanolic solution is due to the presence of aldehyde groups, and a direct relationship exists between the adsorption index at the maximum and the concentration of gossypol in solution [2], which permits the amount of aldehyde groups in a mixture to be determined in a short time with fairly high accuracy (0.1-2%) with the aid of a calibration graph.

In view of this, we have calculated the parameters of the linear calibration graph by the method of least squares. The equation of the straight line reflecting the linear dependence of the optical density on the concentration of gossypol in ethanolic solution has the form $C = 0.032\ D$.

Thus, having calculated the concentration of gossypol in solution from the value of the optical density and knowing the initial concentration taken it is possible to determine the amount of aldehyde groups in the mixture.

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