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SYNTHESIS OF (*bis*-DIMETHYLAMINOETHYLIMINO)GOSSYPOL AND ITS ASSOCIATE WITH POLYVINYLPYRROLIDONE AND THEIR EFFECT ON PROTEIN BIOSYNTHESIS IN RAT LIVER WITH ACUTE HEPATITIS

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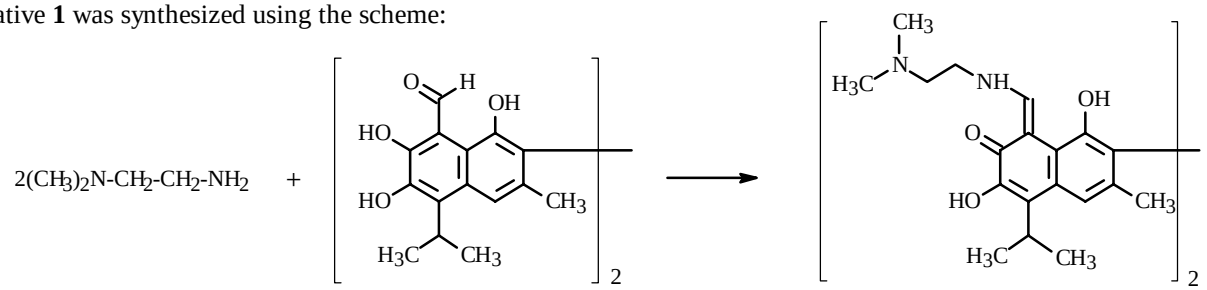
(Bis-dimethylaminoethylimino)gossypol and its associate with PVP were synthesized. Their effects on in vitro and in vivo synthesis of cytoplasmic proteins in rats with acute heliotrinic hepatitis were studied. It was found that heliotrine intoxication substantially suppresses the incorporation of labeled amino acids into cytoplasmic proteins. Administration of gossypol derivatives restored markedly the activity of the hepatocytic protein-synthesizing system.

Key words: gossypol derivatives, protein biosynthesis, hepatitis, heliotrine.

The search for compounds capable of protecting and restoring the functional activity of hepatocytes is currently of great interest.

Our goal was to investigate the synthesis of gossypol derivatives with N,N-dimethylaminoethylamine, (*bis*-dimethylaminoethylimino)gossypol (**1**), and its associate with polyvinylpyrrolidone (PVP, **2**) and to study their effects on cytoplasmic protein biosynthesis of liver with acute hepatitis (AH) induced by administration of heliotrine [1]. Gossypol derivative **1** was synthesized using the scheme:



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The treatment agents were **1** and **2**. The effects of various doses (2-20 μg) of these compounds on protein synthesis in isolated hepatocytes were investigated *in vitro* (Fig. 1a). Administration to the acellular protein-synthesizing system of rat liver cells with AH revealed that **2** increases more effectively the incorporation of labeled amino acids into proteins. As it turned out, 14 μg of **2** not only restored the suppressed protein biosynthesis to the control level but also increased it by 22%. In contrast with **2**, **1** had no noticeable effect on protein synthesis. This may be due to its poor solubility in water and, consequently, its poor availability. Analogous results were observed in *in vivo* experiments (Fig. 1b). The stimulation of protein biosynthesis was greatest with administration of **2** over five days. Administration of **2** over seven days also stimulated incorporation of labeled amino acids into protein but less significantly. The biological activity was determined as before [1-3].

Thus, the molecular complex **2** synthesized by us has a high stimulating effect on protein biosynthesis in rat liver with acute experimental heliotrinic hepatitis because it is biologically more available than **1**.

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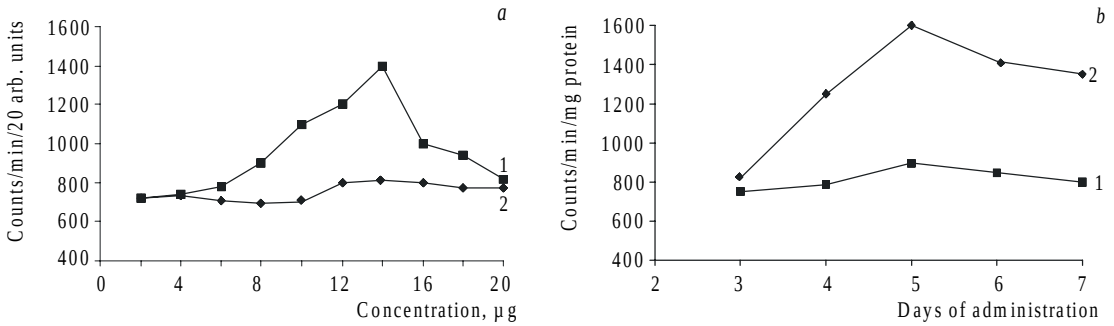

Fig. 1. Effect of various concentrations of **1** and **2** on protein synthesis in rat liver with AH *in vitro* (*a*) and *in vivo* (*b*).

EXPERIMENTAL

PMR spectra were recorded on an XL-100 (Varian, USA) NMR spectrometer at working frequency 100 MHz in $CDCl_3$ with TMS internal standard.

Synthesis of (*bis*-Dimethylaminoethylimino)gossypol (1). Gossypol (0.01 M, 0.518 g) dissolved in ether (100 mL) was vigorously stirred and treated dropwise over 2 h with N,N-dimethylaminoethylamine (0.02 M, 0.176 g) dissolved in ether (20 mL). The resulting precipitate was filtered off and washed several times with ether. The precipitate is a yellow compound that is soluble in $(CH_3)_2CO$, $CHCl_3$, and DMF; slightly soluble in hydrocarbons and ether; and insoluble in water; mp 221°C (dec.); yield 71%; R_f 0.51 (Silufol plates, C_6H_6:$CH_3CO_2C_2H_5$:CH_3OH (7:1:2).

PMR (δ, ppm, J/Hz): 1.43 (6H, d, J = 6.1, 13-CH_3 and 14-CH_3), 2.04 (3H, s, 3-CH_3), 2.25 (6H, d, J = 6.0, 2×NCH_3), 2.70-2.45 (4H, m, NCH_2CH_2N), 3.34 (1H, m, 12-H), 7.74 (1H, s, 4-H), 9.48 (1H, br.d, J = 9.3, 15-H), 14.2 (1N, br.s, NH).

Preparation of the Molecular Complex of (*bis*-Dimethylaminoethylimino)gossypol with PVP (MW = 12,600 ± 2,700) (2). PVP (9 parts) and **1** (1 part) were treated with $CHCl_3$ (250 mL) and stirred for 24 h at room temperature. The solvent was removed. The solid was dried in a thermostatted vacuum chamber at 60-65°C. Yield 97%.

Biological Activity. White mongrel rats (100-120 g) were used in the experiments. AH was induced by a single s.c. administration of heliotrine at 25 mg/100 g body weight. For *in vitro* experiments, an S_{30} fraction containing all components of the protein-synthesizing apparatus (ribosomes, protein translation factors, m-RNA, and aminoacyl-t-RNA-synthetases) was isolated as before [4].

The incubation medium for carrying out the acellular protein synthesis contained the S_{30} fraction; unlabeled amino acids (0.03 μm/mL); a mixture of equally labeled ^{14}C-amino acids (0.01 μCi/mL); a reaction mixture (μmol/mL) of ATP (0.5), GTP (0.05), phosphoenolpyruvate (10), β-mercaptoethanol (5), $MgCl_2$ (15.0), KCl (70), and Tris-HCl (50); pH 7-8; pyruvatekinases (16 μg); and various concentrations (2-20 μg) of **1** and **2**.

In *in vivo* experiments, rats with AH were administered **1** and **2** for 3, 5, and 7 d at concentrations of 0.74 mg and 0.4 mg, respectively, per 100 g of body weight. Four hours before sacrificing, control and experimental rats were injected with ^{14}C-leucine (0.4 μCi/g body weight). Polyribosomes were isolated from the liver by the literature method [1]. The radioactivity of the samples was counted on a Rac-Beta 1217 (LKB, Sweden) counter. The protein content was determined by the literature method [1]. The results were processed using variational statistics on an IBM personal computer using a special program set.

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