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AZO-DERIVATIVES OF GOSSYPOL AND ITS IMINES

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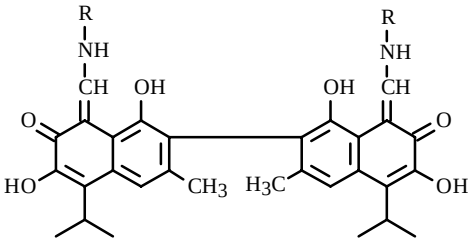
Azo-coupling of diazotized aromatic amines with gossypol and gossypolimines was studied. The physicochemical properties and interferon-inducing activity of the products were determined. It was found that the interferon-inducing activity depended on the reaction type, the position of functional groups (o-, m-, p-) in the added substituents, and the dose and contact time of the compounds with the cells.

Key words: azo-derivatives of gossypol, azo-derivatives of gossypolimines, interferon-inducing activity.

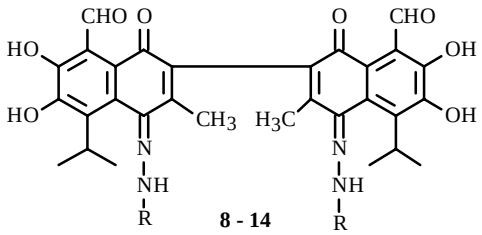
Studies of many gossypol (GP) derivatives found that their biological activity depended on the nature of the substituent. Practically inactive di-, tetra-, and hexaesters were prepared by esterification of GP hydroxyls. Compounds with antiviral, immunomodulating, and interferon-inducing activities were produced by condensation of the aldehydes with amines and compounds with reactive methylenes. Preparations with antiherpetic (megosin), immunosuppressive (batriden), and antichlamydic (gosalidon) activities and an interferon inductor (ragosin) were based on some of these.

We studied the biological activity of GP derivatives prepared by azo-coupling with various diazotized amines at C-4 of GP. The interferon-inducing activities were analyzed and compared as a function of the site of addition of the amine to the aldehyde groups and to C-4.

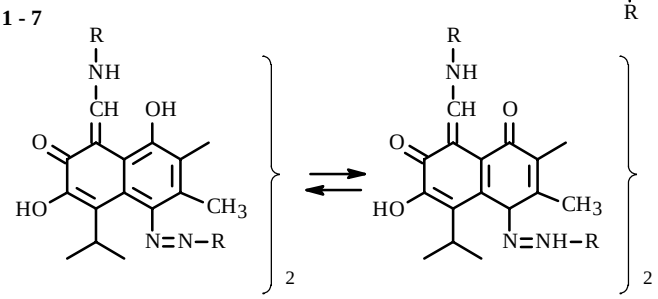
We synthesized GP imines (**1-7**) and GP azo-derivatives (**8-14**) with the same arylamines (Table 1).



1 - 7



8 - 14



15 - 21

where R = **1, 8, 15:** = -C_6H_5;
2, 9, 16: = -C_6H_4OH-2; **5, 12, 19:** = -C_6H_4COOH-2;
3, 10, 17: = -C_6H_4OH-3; **6, 13, 20:** = -C_6H_4COOH-3;
4, 11, 18: = -C_6H_4OH-4; **7, 14, 21:** -C_6H_4COOH-4

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TABLE 1. Properties of Azo-Derivatives of Gossypol **8-21**

Compound	mp, °C	R_f	UV spectrum (acetone), λ_{max}, nm, (log ε)	Empirical formula	Yield, %
8	153-155	0.55	440 (4.15)	$C_{42}H_{38}N_4O_8$	58.84
9	298-300	0.65	445 (4.75)	$C_{42}H_{38}N_4O_{10}$	54.08
10	293-295	0.60	420 (4.22)	$C_{42}H_{38}N_4O_{10}$	53.23
11	296-298	0.58	425 (4.48)	$C_{42}H_{38}N_4O_{10}$	64.25
12	> 350	0.71	460 (4.71)	$C_{44}H_{38}N_4O_{12}$	80.12
13	> 350	0.69	455 (4.63)	$C_{44}H_{38}N_4O_{12}$	83.20
14	> 350	0.67	465 (4.79)	$C_{44}H_{38}N_4O_{12}$	78.31
15	210-212	062	455 (4.14)	$C_{54}H_{48}N_6O_6$	62.20
16	310-312	0.61	465 (4.16)	$C_{54}H_{50}N_6O_{10}$	72.31
17	320-323	0.53	460 (4.17)	$C_{54}H_{50}N_6O_{10}$	67.01
18	310-311	0.65	460 (4.16)	$C_{54}H_{50}N_6O_{10}$	72.83
19	> 360	0.67	470 (4.16)	$C_{58}H_{48}N_6O_{14}$	78.92
20	> 360	0.71	465 (4.15)	$C_{58}H_{48}N_6O_{14}$	81.30
21	> 360	0.69	475 (4.17)	$C_{58}H_{48}N_6O_{14}$	63.28

The physicochemical properties of **1-7** have been described previously [1].

The structures of **1-14** were proved by PMR.

The PMR spectrum of **1** contains a doublet for the methyls at 1.54 ppm. A singlet at 2.13 ppm corresponds to the methyl on C-3; a multiplet at 3.70 ppm, the methine proton of the isopropyl group. The five aromatic protons of the substituent give a structureless signal at 7.1-7.5 ppm. A singlet at 7.68 ppm corresponds to the aromatic proton on C-4. In contrast with the spectrum of starting GP, that of **1** lacks a signal for the aldehyde proton at 11.2 ppm and exhibits a doublet at 10.25 ppm (J = 10 Hz). The chemical shift and the doublet for the C-15 proton are consistent with the quinoid structure for **1**.

All lines in the PMR spectrum of **8** are distinctly broadened, apparently due to dynamic processes and the presence of an unpaired electron because **8-14** in the solid state have well resolved EPR signals. The signal for the aldehyde proton at 11.2 ppm, the lack of a signal for the C-4 proton, the constancy of the other signals, and the results from a previous study of the azo-coupling products of GP with sulfanilamide preparations [2] lead to the conclusion that **8-14** exist primarily as the quinoid forms.

In continuation of research on N-containing GP derivatives, we synthesized azo-derivatives of GP imines **15-21**, the physicochemical properties of which are listed in Table 1.

All lines in the PMR spectrum of **15** are broad, like in that of **8**. The doublet of the ketoimine proton at 10.2 ppm is substantially broadened and consistent with the quinoid structure of ring A. It is known [3, 4] that hydroxyazo—quinonehydrazo tautomerism is characteristic for azo-derivatives of naphthols. The relative fraction of each form can be estimated by calculations. Molecular mechanics MM^+ in the integrated calculation set HyperChem were used to calculate **15**. The heat of formation was 109.8 kcal/mol for the quinoid form and 115.8 kcal/mol for the benzoid form. The gain of 6 kcal/mol in the heat of formation indicates the preferential (>99.0%) existence of **15** in the quinonehydrazo form (at room temperature).

No compounds inducing high titers of interferon were found among **1-14** (Fig. 1). The interferon-inducing activity of them was less than 80-160 IE units/mL. However, **15-21** exhibited interferon-inducing activities of 320-1280 IE units/mL. Therefore, it can be assumed that the activity depends to a certain extent on the number of added substituents and the type of reaction used to synthesize the compound.

The most interesting results were obtained for compounds prepared by substituting both the aldehyde and C-4.

Comparison of the interferon-inducing activities of **15-21** as functions of the position (*o*-, *m*-, *p*-) of functional groups added to the aldehydes and C-4 showed that the activity was highest mainly for the *o*-isomers (**16** and **19**). The least active compounds were synthesized using *m*-aminobenzoic acid and *m*-aminophenol.

Compound **19**, which induced 1280 IE units/mL, is the most interesting for further research.

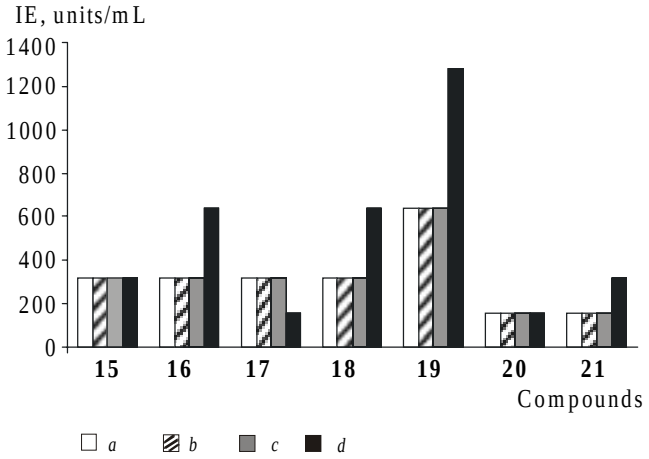

Fig. 1. Interferon-inducing activity of **15-21**. Dose 100 mg/kg, after 24 h (a), 100 mg/kg, 48 h (b), 200 mg/kg, 24 h (c), 200 mg/kg, 48 h (d).

EXPERIMENTAL

The identity and purity of the prepared compounds were monitored by TLC on Silufol UV-254 plates using acetone:toluene (6:4) with development by fluoroglucinol (1%) in HCl (2 N) in ethanol. Elemental analyses corresponded to those calculated. UV spectra were recorded on a SF-26 spectrophotometer (c = 0.002%). PMR spectra were recorded on an XL-100 (Varian, USA) NMR spectrometer at working frequency 100 MHz in mainly DMSO-d_6 but also $CDCl_3$.

Preparation of 8.

a. Diazotization of Aniline. A solution of aniline (freshly distilled, 0.18 g, 0.02 M) in water (5 mL) and conc. HCl (2 mL) was cooled to 0-2°C and diazotized by $NaNO_3$ solution (30%, 0.7 g, 0.1 M). The reaction mixture for diazotization should remain acidic (Congo dye). After completion of the diazotization, the contents are stirred for another 15 min and very carefully neutralized with NaOAc until the acidic reaction almost disappears.

b. Azo-coupling. A solution of the diazonium salt resulting from the diazotization of aniline was stirred, cooled to -2°C, and added quickly and dropwise to an alcoholic solution of GP (0.52 g, 0.001 M). The completion of the reaction was monitored by β-naphthol in alcohol. The resulting precipitate was filtered off, washed with diethylether, and dried. Yield 0.41 g (58.84%), mp 153-155°C, R_f 0.55, brick-red powder, soluble in DMSO and DMF, poorly soluble in organic solvents, practically insoluble in water.

Compounds **9-14** were synthesized by the same method.

Preparation of 15. A solution of **8** was treated with a solution obtained by heating aniline (0.18 g, 0.02 M) in ethanol. The color of the reaction mixture changed to dark red. Heating was continued for 20-25 min. The solution was cooled. After 15-20 min a dark red precipitate formed. It was filtered off, washed with hexane, and dried to yield a dark red powder that was soluble in DMSO and DMF; poorly soluble in alcohols, acetone, and diethylether; and practically insoluble in water.

Compounds **16-21** were prepared by the same method.

The interferon-inducing activities of **1-21** were determined in white mongrel mice of mass 10-12 g. The preparations were administered once intraperitoneally at doses of 100 and 200 mg/kg. The interferon content was determined by titration of serum in homologous culture cells using the degree of protection against the cytopathologic action of mouse encephalomyocarditis virus after 24 and 48 h.

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