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SYNTHESIS AND INTERFERON-INDUCING ACTIVITY OF AZO-DERIVATIVES OF GOSSYPOL AND ITS IMINES

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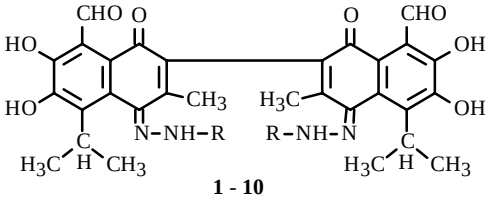
New azo-derivatives of gossypol and its imines were described. Their physicochemical properties and data on their interferon-inducing activity were presented.

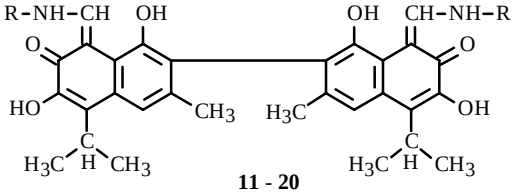
Key words: azo-derivatives of gossypol, azo-derivatives of gossypol imines, interferon-inducing activity.

One of the features of gossypol (GP) derivatives is the ability of some of them to induce the formation of high interferon titers in man [1, 2]. Interferon inducers are found among the azomethines (megosinum and ragosin) and condensation products of GP with methylene-active compounds (batridenum). It has seemed interesting to determine if C-4 azo-derivatives of GP have this capability. Sulfanilamide preparations, which are known to have a wide spectrum of physiological activity, were used as the azo-compounds [3].

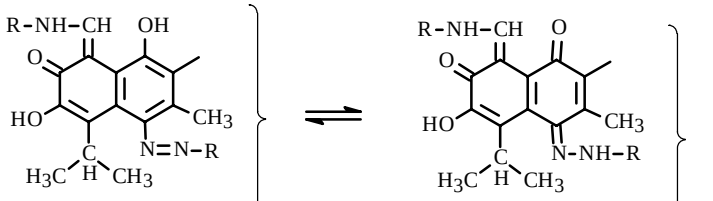
We synthesized new azo-derivatives of GP (**1-10**). Table 1 gives their physicochemical properties [4].



1 - 10



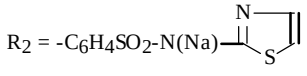
11 - 20



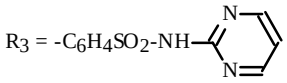
21 - 30

where R = R_1 - R_{10}

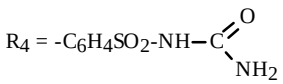
R_1 = -$C_6H_4SO_2$-NH_2



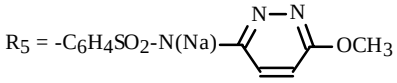
R_2 = -$C_6H_4SO_2$-N(Na)



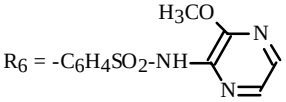
R_3 = -$C_6H_4SO_2$-NH



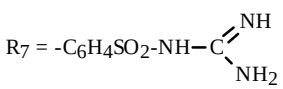
R_4 = -$C_6H_4SO_2$-NH-C(=O)-NH_2



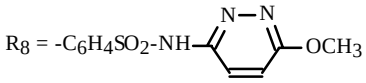
R_5 = -$C_6H_4SO_2$-N(Na)-OCH_3



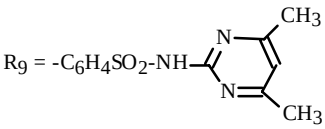
R_6 = -$C_6H_4SO_2$-NH-OCH_3



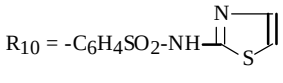
R_7 = -$C_6H_4SO_2$-NH-C(=NH)-NH_2



R_8 = -$C_6H_4SO_2$-NH-OCH_3



R_9 = -$C_6H_4SO_2$-NH-CH_3



R_{10} = -$C_6H_4SO_2$-NH-thiazole

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TABLE 1. Physicochemical Properties of Azo-Compounds of Gossypol (**1**-**10**)

Compound	R	mp, °C	UV spectrum, nm, λ_{max} (log ε), acetone	Empirical formula	Yield, %	Interferon-inducing activity IU (units/mL) after			
						24 h	48 h	24 h	48 h
						100 mg/kg		200 mg/kg	
1	R_1	248-50	490(4.76)	$C_{42}H_{40}N_6O_{12}S_2$	48.17	20	20	40	80
2	R_2	>350	460(4.17)	$C_{48}H_{40}N_8O_{12}Na_2S_4$	55.57	80	160	160	160
3	R_3	277-78	460(4.16)	$C_{50}H_{44}N_{10}O_{12}S_2$	64.1	20	20	20	20
4	R_4	279-81	460(4.16)	$C_{44}H_{42}N_8O_{12}S_2$	66.30	20	20	40	80
5	R_5	220-22	475(4.17)	$C_{52}H_{46}N_{10}O_{14}Na_2S_2$	60.35	160	160	160	160
6	R_6	224-26	460(4.46)	$C_{52}H_{48}N_{10}O_{14}S_2$	74.70	20	20	80	80
7	R_7	285-87	465(4.20)	$C_{44}H_{44}N_{10}O_{12}S_2$	53.68	20	20	40	40
8	R_8	298-300	450(4.58)	$C_{52}H_{48}N_{10}O_{14}S_2$	67.84	40	20	80	40
9	R_9	215-18	485(4.54)	$C_{54}H_{52}N_{10}O_{12}S_2$	44.10	20	20	20	10
10	R_{10}	275-77	470(4.76)	$C_{42}H_{42}N_8O_{12}S_4$	54.08	10	20	20	20

TABLE 2. Interferon-Inducing Activity of Gossypol Imines (**11**-**20**)

Compound	R	Interferon-inducing activity IU (units/mL) after			
		24 h	48 h	24 h	48 h
		100 mg/kg		200 mg/kg	
11	R_1	20	20	20	40
12	R_2	40	40	80	80
13	R_3	10	10	10	10
14	R_4	10	10	20	40
15	R_5	80	40	80	80
16	R_6	20	20	20	20
17	R_7	10	10	10	10
18	R_8	80	160	320	160
19	R_9	10	10	20	20
20	R_{10}	20	40	40	80

It can be seen that the interferon-inducing activity (IA) depends on the nature of the substituent and is dose-dependent and time sensitive. A substituent effect was also found for the IA of GP imines (**11**-**20**) with the same R_1-R_{10} substituents [5]. According to Table 2, the IA for **11**-**20** also depends on the substituent on the aldehyde, the dose, and the duration of action. The IA is low, not exceeding 160 IE (units/mL), with substituents only on C-4 or the aldehydes (Tables 1 and 2).

Compounds **21**-**30** were synthesized to determine if the IA of the GP azo-derivatives prepared via azo-combination of **1**-**10** with **11**-**20** would change. Table 3 lists their properties.

The UV spectra of **21**-**30** showed that the most characteristic feature, like for **1**-**10**, is absorption in the range 460-490 nm. The study of the PMR spectra of **1**-**30** is complicated by the exceedingly low solubility in most organic solvents. The lines of spectra recorded in DMSO are significantly broadened.

Nevertheless, it can be noted that the PMR spectra are qualitatively and quantitatively consistent with the proposed structures. For example, the spectra of **9**, **19**, and **29** have distinct signals for the isopropyl methyls of GP at 1.45 ppm and for the sulfadimesine methyls at 2.24 ppm. The integrated intensities of these signals indicate unambiguously that **9** and **19** each have two substituents on GP whereas **29** has four. For **8**, **18**, and **28**, the integrated intensities of the GP isopropyl methyls (1.45 ppm) and the sulfapyridazine hydroxymethyl (3.92 ppm) are also consistent with the spectra of the proposed structures.

TABLE 3. Physicochemical Properties of Azo-Derivatives of Gossypol Imines (**21**-**30**)

Compound	R	mp, °C	UV spectrum, nm, λ_{max} (log ε), acetone	Empirical formula	Yield, %	Interferon-inducing activity IU (units/mL) after			
						24 h	48 h	24 h	48 h
						100 mg/kg		200 mg/kg	
21	R_1	306-08	465(4.18)	$C_{54}H_{54}N_{10}O_{14}S_4$	63.71	80	160	160	160
22	R_2	>360	475(4.23)	$C_{66}H_{54}N_{14}O_{14}Na_4S_8$	73.70	320	320	320	320
23	R_3	>360	465(4.22)	$C_{74}H_{66}N_{18}O_{18}N_4S_4$	68.91	320	320	320	640
24	R_4	272-74	470(4.33)	$C_{74}H_{70}N_{18}O_{14}S_4$	67.63	80	160	160	320
25	R_5	302-04	475(4.25)	$C_{58}H_{54}N_{18}O_{14}S_4$	70.18	80	80	160	160
26	R_6	278-80	465(4.21)	$C_{76}H_{74}N_{12}O_{16}S_4$	61.30	320	160	640	320
27	R_7	293-95	474(4.20)	$C_{70}H_{62}N_{12}O_{14}S_4$	59.43	20	40	40	80
28	R_8	288-90	485(4.20)	$C_{66}H_{58}N_{12}O_{14}S_8$	63.27	80	160	160	160
29	R_9	293-95	474(4.20)	$C_{70}H_{62}N_{12}O_{14}S_4$	59.43	20	40	40	80
30	R_{10}	288-90	485(4.20)	$C_{66}H_{58}N_{12}O_{14}S_8$	63.27	80	160	160	160

The IA of **1**-**30** was determined in vitro. The results show that the IA is clearly dose- and time-dependent and depends on the nature and number of substituents.

Compounds **22**, **25**, and **28** have the highest IA. These are azo-derivatives of GP imines, the study of which will be continued.

EXPERIMENTAL

The purity and spectral properties of the compounds were determined as before [5].

Di{*p*-aminobenzenesulfamido)-4,6-di-4′-azo-[2′-(8-formyl-1′,6′,7′-trihydroxy-3′-methyl-5′-isopropyl]-naphthalene} (9). a) A cooled (0-2°C) solution of sulfadimesine (0.560 g, 0.002 mole) in water (5 mL) and conc. HCl (1.2 mL) was treated with $NaNO_2$ (3.5 g, 0.05 mole, 30% solution). The reaction medium should remain acidic during the diazotization (by Congo). After the reaction was finished, the contents were stirred for another 15 min and carefully neutralized by NaOAc (by Congo).

b) A solution of diazonium salt prepared by diazotization of sulfadimesine (a) was stirred, cooled (-2°C), and treated rapidly and dropwise with GP (0.52 g, 0.001 mole) in alcohol. The reaction was monitored using β-naphthalene in alcohol. The precipitate was filtered off, washed with diethyl ether, and dried.

Yield of **9**, 0.47 g (44.1%) of brown amorphous solid, mp 215-218°C, R_f 0.68 (acetone:toluene, 6:4). Compounds **1**-**10** were prepared by the same method.

Preparation of 29. A solution of **9** was treated with a solution prepared by heating sulfadimesine (0.28 g, 0.02 mole) in ethanol. The reaction mixture became dark brown. Heating was continued for 20-25 min. The solution was cooled. After 15-20 min, a dark brown precipitate began to form. This was filtered off, washed with hexane, and dried.

Yield of **29**, 0.56 g (59.43%), mp 293-295°C, R_f 0.72 (acetone:toluene, 6:4). Compounds **21**-**30** were synthesized by this same method.

The IA of **1**-**30** was determined using generic white mice of mass 10-12 g. The preparations were administered once i.p. at doses of 100 and 200 mg/kg. The interferon content was determined by titration of serum with cells of a homologous culture using the degree of protection from the cytopathic action of mouse encephalomyocardium virus after 24 and 48 h.

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