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NEW AZO DERIVATIVES OF GOSSYPOL

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New azo derivatives of gossypol are described. Their physicochemical properties are reported. The presence of hydroxyazo- and quinonhydrazo-tautomers is proposed.

Key words: gossypol, azo derivatives.

Various types of organic dyes are used in medical practice [1]. These include triphenylmethanes (brilliant green), thiazines (methylene blue, chlorpromazine), and acridines (acrikhim, tripaflavine, rivanol) [2]. However, azodyes, the most numerous and most important class of organic dyes, have practically no known applications in medicine.

Keeping this in mind and considering that phenols and naphthols are frequently one of the components of azo-coupling reactions, we used the polyfunctional polyphenol gossypol (GP) for azo-coupling reactions.

Certain azo derivatives of GP have been previously prepared [3, 4]. It was shown that they can be used as azodyes [5].

We used diazotized aliphatic and aromatic amines and certain sulfanilamides for the coupling with GP. The azo derivatives of GP are brightly colored finely crystalline compounds that are poorly soluble in organic solvents and practically insoluble in water.

Azo derivatives of phenols are known to exist primarily in the hydroxyazo form. The quinonhydrazo form is characteristic of arylazo derivatives of anthrols and phenanthrols. Both forms can exist for naphthols [6, 7]. The conclusion that an azo—hydrazo tautomerism exists was made mainly from studying IR spectra of 1-arylazo-2-naphthols and 4-arylazo-1-naphthols [7, 8]. It was noted that the equilibrium shifts to the side of the hydrazone in polar solvents and under the influence of electron-accepting substituents in the aryl group [9, 10]. The question about the existence of tautomeric equilibrium of substituted naphthols was finally resolved using NMR spectra of various arylazonaphthols, in which both atoms of the azo group were N^{15} isotopes [6, 10, 11].

On this basis, the existence of tautomeric hydroxyazo—quinonhydrazo equilibrium can be proposed for azo derivatives of GP with the dinaphthalene structure. If both forms of the compound are in equilibrium (at constant concentration and temperature), then the UV spectra of their solutions that are recorded in solvents of different polarity should produce plots that intersect at a point where both forms have identical absorption coefficients. The appearance of an isosbestic point would prove experimentally that two equilibrium forms exist, as noted previously [12].

We recorded UV spectra of **14** in solvents of different polarity (methanol and CCl_4). The presence of an isosbestic point (Fig. 1) indicates that equilibrium forms of this compound exist. Such a phenomenon was also observed for **3** and **5** in these same solvents. An isosbestic point was observed at 325 nm ($D = 0.22$) and 390 nm ($D = 0.23$), respectively.

Thus, the results of the UV spectroscopic study suggest that tautomeric equilibrium of I—II occurs for azo derivatives of GP (Fig. 2).

During the IR spectroscopic study of azo derivatives of GP, we compared the spectra of GP; the dimethylether of GP (DMEG); and the quinone of GP (gossipolone, GPP), for which ring B has the quinoid structure; the condensation product of 4-amino-(1-phenyl-2,3-dimethyl)-5-pyrazolone (ANTP) with GP at the aldehydes [13], for which it has been proven that ring A has the quinoid form and ring B has the benzoid form (**15**); and azo-coupling products of ANTP at C_4 with GP (**14**) and DMEG (**16**).

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TABLE 1. Absorption Bands in IR Spectra of Studied Compounds

Vibration type	Gossypol	Gossypolone	4-Aminoantipyrine	Compound			DMEG
				14	**15**	**16**	
ν(OH)	3500, 3410	3470		3500		3270	3250
ν(NH)			3330, 3400	3330	3330		
ν(C=O)			1640	1640	1640	1640	
ν(C=N)						1750	
ν (C=O and conj. systems)	1610	1625	1600	1630			1590
Benzene vibrations			1500	1500	1500		
β(COH), ν(C-O)	1350				1310		1290
ν(C-O), β(COH)	1180				1170		990, 840
$\phi(CH_3)$		1300		1300			

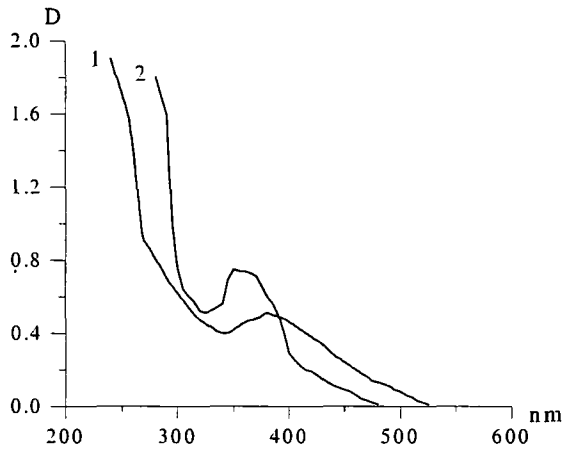

Fig. 1

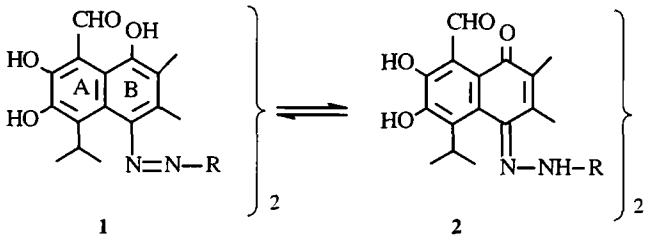

Fig. 2

Fig. 1. UV spectrum of compound **14** in methanol (1) and CCl_4 (2).
Fig. 2. Tautomers of gossypol azo derivatives.

The IR spectrum of GP (Table 1) contains absorption bands at 3500, 3410, and 3300 cm^{-1} that arise from hydroxyl stretching vibrations and a doublet at 1610 cm^{-1} that is due to combination vibrations of carbonyls and the conjugated-bond system. In the IR spectrum of GPP, the last absorption band is observed at 1625 cm^{-1}. This is explained by the decreased degree of conjugation in GPP (ring B), which exists in the quinoid form, compared with the benzoid structure of GP. Stretching vibrations of GPP hydroxyls appear as one absorption band at 3470 cm^{-1}.

Absorption bands are observed at 1640 and 1500 cm^{-1} in the IR spectrum of the enamine of GP (**15**). These arise from vibrations of carbonyls and the benzoid fragment of ANTP, respectively. The intensity of the band at 1640 cm^{-1} compared with that at 1500 cm^{-1}, which changes little, decreases on going from ANTP to **15**. This is explained by a decrease in the polarity of the C=O group in ANTP owing to the formation of the enamine **15**.

An absorption band at 1630 cm^{-1} is observed on the background of poorly resolved absorption bands at 1640 and 1500 cm^{-1} in the IR spectrum of the GP azo derivative (**14**). This band is assigned to vibrations of the aldehyde groups of GP. The presence of this absorption band, like for GPP, might indicate the quinoid structure of ring B in **14**.

The IR spectrum of **16** contains an absorption band at 1640 cm^{-1} that is due to vibrations of the C=O groups in ANTP. A neighboring band at 1590 cm^{-1} is assigned to vibrations of GP aldehyde groups. This is consistent with the benzoid structure of ring B in **14**. The presence of an absorption band at 1750 cm^{-1}, which is absent in the IR spectra of **14** and **15**, may be due to the presence of a >C=N– bond, which, in turn, also is consistent with the benzoid structure of ring B in **16**.

The low solubility of the new azo derivatives of GP in most organic solvents at present unfortunately prevents their study by NMR spectroscopy.

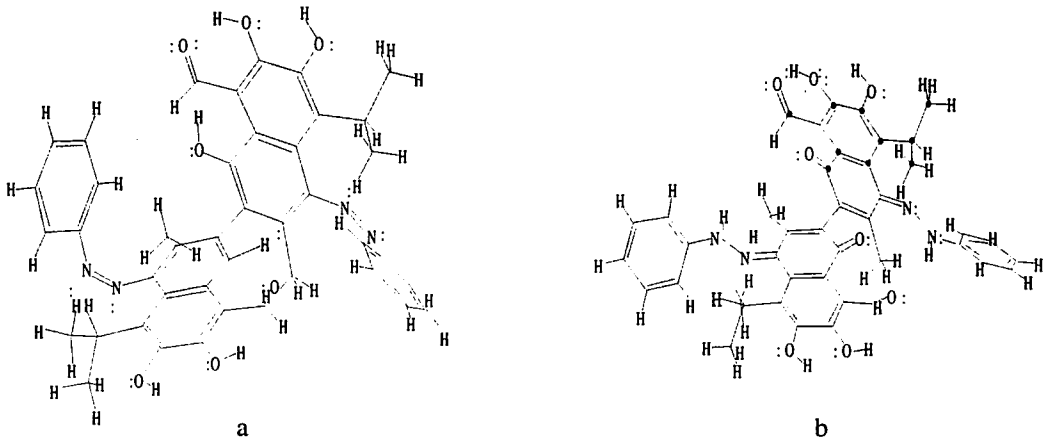

Fig. 3. Molecular models of compound **3**: hydroxyazo (a) and quinonhydrazo (b) forms.

In addition to the physicochemical methods for studying the structure of azo derivatives of GP, we used molecular modeling of the two possible tautomeric forms based on **3**. The study used the PCMODE-MMX molecular mechanics method. The models were constructed using substituted naphthyl fragments of the same structure, which exist in **3** in the two possible tautomeric forms.

The calculated geometric parameters during the construction of the model of **3** were within the range determined earlier for GP [14] and its derivatives [15].

In particular, the length of the C_2–C_2' bridge was 1.49 Å for the azo form and 1.48 Å for the hydrazo form after optimization of the structures. The dihedral angles of the bonds to the bridge were 48, 26, 40, and 17°. Keeping in mind the presence of bulky substituents in **3**, these data are within the range determined for GP itself.

The total energies of the molecules in the two probable tautomeric forms are E_{tot} = 285.36 kcal/mol (azo form) and E_{tot} = 194.69 kcal/mol (quinonhydrazo form). This indicates that the quinonhydrazo form II is preferred (Fig. 3).

Thus, the results of the UV and IR spectroscopic studies and the molecular modeling suggest that azo derivatives of GP exist in hydroxyazo- and quinonhydrazo-tautomeric equilibrium in solutions and primarily in the quinonhydrazo form in the solid state.

Studies of the biological activity of the azo derivatives of GP revealed for several of them interferon-inducing activity of 320-640 units/ml at a dose of 100 mg/kg.

Further studies are planned.

EXPERIMENTAL

TLC of the GP azo-coupling products was performed on Silufol UV-254 plates using acetone—toluene (6:4). UV spectra were recorded on a SF-26 spectrophotometer (C = 0.002%); IR spectra, on a Specord 71-UR spectrophotometer as mulls in mineral oil. Elemental analyses of the synthesized compounds agreed with those calculated.

Di-{4-amino-(1-phenyl-2,3-dimethyl-5-pyrazolon)-4′-azo-[2′-(8′-formyl-1′,6′,7′-trihydroxy-3′-methyl-5′-isopropyl]-naphthalene} (14). A solution of the diazonium salt, prepared by diazotization of ANTP (0.4 g, 2 mmole) according to the literature method [16], was added rapidly and dropwise with stirring and cooling to -2°C to a solution of GP (0.52 g, 1 mmole) in alcohol. The course of the reaction was monitored using β-naphthol in alcohol. The precipitate was filtered off, washed with diethylether, and dried. Yield 0.74 g (80.0%) of an amorphous orange solid, mp 279-281°C, R_f 0.80. UV spectrum (λ_{max}, acetone, nm): 380 (log ε 4.73).

Qualitative reactions with $FeCl_3$ (solution turns red) and $NaNO_3$ (yellowish-green color) confirmed the formation of the azo compound [17].

1,1′6,6′-Tetrahydroxy-5,5′-diisopropyl-3,3′-dimethyl-7,7′-dioxo-8,8′-dimethyl-4″,4′″-diimino-(2″,2′″,3″,3′″-tetramethyl-5″,5′″-dipyrazolon)-2,2″-dinaphthalene (15). A solution of GP (1.04 g, 2 mmole) in diethylether (20 ml) was

treated with 4-N-(1-phenyl-2,3-dimethyl-5-pyrazolone) (0.81 g, 4 mmole) in ethanol (5 ml) and heated on a water bath for 2 h. The mixture of precipitates that formed on cooling was filtered off, washed with ethanol and diethylether, and dried at 60-65°C. Yield 1.26 g (70.8%) of an orange powder, mp 254-256°C, R_f 0.70 (acetone—toluene, 6:4). UV spectrum (λ_{max}, $CHCl_3$, nm): 440, 465 (log ε 4.12, 4.09).

GPP and DMEG were prepared according to the literature methods [18, 19].

Preparation of Compound 16. A solution of the diazonium salt obtained by diazotization of 4-amino-(1-phenyl-2,3-dimethyl-5-pyrazolone) (0.4 g, 2 mmole) [16] was added quickly and dropwise with stirring to a solution of DMEG (0.57 g, 1 mmole) with cooling to -(2-3)°C. The course of the reaction was monitored using β-naphthol in alcohol. The precipitate was filtered off and washed with diethylether. Yield 0.63 g (66.6%) of an amorphous light yellow compound, mp 192-194°C, R_f 0.60. UV spectrum (λ_{max}, acetone, nm): 345 (log ε 4.05).

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