Spectroscopic and Photochemical Studies of Gossypol in Solution

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Spectroscopic properties of gossypol and its stability in different solvents and pH are studied by means of UV-visible absorption and emission spectroscopy. The results are correlated and discussed on the basis of semiempirical INDO/S CI calculations. The influence of oxygen and excitation wavelength on the photodecomposition of gossypol in solution is also investigated.

Introduction

Gossypol, 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl(2,2′-dinaphthalene)-8,8′-dicarboxaldehyde (Fig. 1) is a yellowish compound present in various parts of cotton plants [1]. The biological importance of gossypol, e.g. contraceptive and toxic activities [1–3], was the reason for many recent extensive studies in solution [4–8]. However, an analysis of the electronic properties of gossypol as well as its photochemical stability in solution were not discussed in great detail. In this paper the spectroscopic and photochemical properties of gossypol in solution are presented.

Experimental Details

Yellow microcrystalline pure gossypol obtained from the Institute of Bioorganic Chemistry, Academy of Sciences of UzSSR, Tashkent, USSR, was recrystallized twice from hexane (m. p. 180–181 °C; Elemental Anal., calc. for $C_{30}H_{30}O_8$ (%): C, 69.5; H, 5.79; found: C, 69.3; H, 5.74). All solvents (except ethanol and water) and reagents were of spectroscopical grade and used without further purification. Water was double distilled from a quartz apparatus. Fluorescence grade ethanol 95% (Merck) was used as received. Silica gel 60 F_{254} silanised pre-coated plates (Merck) with $CHCl : (CH_3)_2CO : HCO_2H$ as solvent system were used for TLC analysis.

UV-visible absorption spectra were recorded using a Specord M-40 (Zeiss) spectrophotometer, and emission and excitation spectra were measured by means of an MPF 66 (Perkin-Elmer) spectrofluorimeter with a low temperature accessory.

The photochemical irradiations were carried out on an optical bench using a high pressure HBO 200 mercury lamp (Narva) with a combination of glass and interference filters to isolate the 366 nm mercury line, or with a low pressure TNN 15/30 mercury lamp (Original Hanau) for $\lambda = 254$ nm. The photochemical studies, deoxygenation and luminescence quantum yield measurements were carried out according to the procedure described in [9].

The electronic properties of gossypol were calculated using a spectroscopically parametrized INDO/S CI method in a similar way to that described previously [10]. Bond lengths and valence angles for the gossypol molecule were taken from the crystallographic study [11].

Results and Discussion

1. Absorption and Emission Properties

The UV-visible absorption spectra of gossypol in solution consists of three well separated bands. The positions of absorption maxima and the values of molar absorption coefficients depend significantly on the solvent used (Table 1). The recorded spectra are similar to those observed in ethanol [12–14], chloroform and cyclohexane [14], and are the same for samples of gossypol recrystallized from different solvents.

As shown in Table 1, an increase of solvent polarity results in a shift of the long-wavelength absorption band III to longer wavelengths. The change of pH

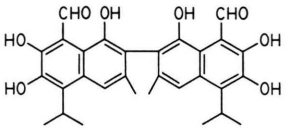

Fig. 1. The molecular structure of gossypol.

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Table 1. Absorption maxima and molar absorption coefficients of gossypol in different solvents ($c \sim 10^{-5}$ M).

Solvent	Band I		Band II		Band III	
	λ_{max} (nm)	ε (M^{-1} cm^{-1})	λ_{max} (nm)	ε (M^{-1} cm^{-1})	λ_{max} (nm)	ε (M^{-1} cm^{-1})
Hexane	236	110 000	286	36 000	357 [a]	20 000
Carbon tetrachloride	–	–	288	33 000	361	19 400
Acetone	–	–	–	–	372	17 500
Acetonitrile	235	63 000	289	30 000	373	14 700
Ethanol	237	82 600	289	31 300	377	17 800
Ethanol (buffered pH = 3.2)	236	94 800	290	33 000	373	17 300
Ethanol (buffered pH = 9.5)	242	76 000	~273	31 000	393	19 000
			297	24 000		
Water (pH = 6.5)	235	68 000	289	25 000	373	14 600
Water (buffered pH = 4.0)	236	–	290	–	373	–
Water (buffered pH = 10)	242	–	~294	–	388	–

[a] A long-wavelength week absorption at $\lambda \sim 430$ nm ($\varepsilon \sim 100$) is additionally observed in a tail of the band III in hexane solution.

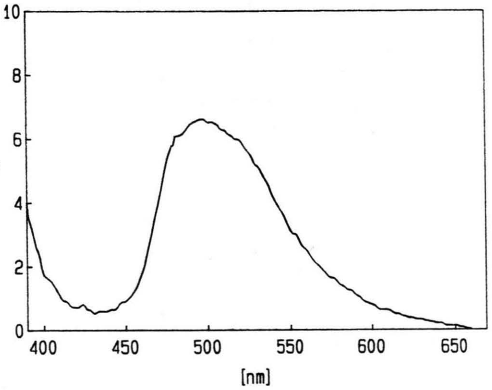

Fig. 2. Emission spectrum of gossypol in ethanol at 77 K ($c = 6 \times 10^{-5}$ M, $\lambda_{ex} = 375$ nm).

affects also the absorption spectra of gossypol. Similarly to *ortho*-hydroxy-naphthaldehyde and its derivatives [15], the anionic form of gossypol is observed in basic solutions (*vide infra*).

The stability of gossypol dissolved in various solvents and at different pH has recently been discussed [4, 14]; it has been shown that gossypol is chemically unstable in solution. However, all gossypol solutions used in this study, except solutions in ethanol at pH < 5, appeared to be relatively stable at room temperature in dark within one hour (less than 5% decomposition after 1 h in dark). In the acid ethanol solution, gossypol is very unstable because of an acid catalysed formation of acetal; e.g. the rate constant of gossypol decomposition in buffered ethanol with pH = 2.5 at room temperature was determined to be $(3.44 \pm 0.04) \times 10^{-5}\ s^{-1}$. The kinetics of this reaction is under investigation and will be presented elsewhere.

Gossypol does not show any luminescence in solution at room temperature ($\Phi_e < 10^{-5}$). However, in the rigid solution at 77 K an emission with a maximum at about 490 nm is observed (Figure 2). This emission can be attributed to the emission from the gossypol molecule due to two observations: a) the excitation and absorption spectra of gossypol are very similar, b) the recrystallization of the gossypol samples has no influence on the emission properties of gossypol. The quantum yield of gossypol luminescence in ethanol at 77 K was determined to be $\Phi_e = 0.015 \pm 0.03$. This low temperature emission may be assigned to a phosphorescence from the lowest excited triplet state of gossypol (Table 2). However, the assignment of this emission to the luminescence of tautomeric forms of gossypol [6] cannot be ruled out.

The observed spectroscopic properties of gossypol can be discussed on the basis of semiempirical INDO/S CI calculations. The calculations were performed for the gossypol molecule and its anionic forms using the bond lengths and valence angles taken from the crystallographic study [11], with various assumed values of the dihedral angle between the naphthalene subunits. The observed and calculated spectra, given in Tables 2 and 3, are in good qualitative agreement. Comparing the results one must bear in mind that the calculated spectra refer to molecules in vacuo, while the observed spectra have been recorded in solutions. Despite of this limitation, there is a good correlation between the observed and calculated changes in the transition energies and oscillator

Table 2. Observed and calculated transition energies and oscillator strengths of gossypol.

Observed		Calculated		
Energy (cm^{-1})	Molar absorption coefficient (M^{-1} cm^{-1})	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
		A	23 300	0.00005
~23 200	~100	B	23 300	0.00009
		A	28 500	0.016
25 600 (sh)	~10 000	B	28 500	0.064
28 000	20 000	B	31 000	0.202
		A	31 200	0.238
35 000	36 000	B	35 500	1.505
		A	36 600	0.023
		B	37 900	0.250
		A	38 100	0.001
		B	38 200	0.064
		A	38 500	0.159
42 400	110 000	B	40 400	1.155
		A	42 000	0.211
		B	42 200	0.272
		B	42 600	0.097
		A	42 700	0.046
		A	43 000	0.306
		B	43 100	0.004
		A	43 100	0.117
Triplet states [b]				
20 400	$< 10^{-3}$ [c]	A	22 500	0.16 [d]
		B	22 500	0.007 [d]
		A	24 800	–
		B	25 100	–

[a] From absorption spectra in hexane.
[b] From emission spectra in ethanol at 77 K, assigned to phosphorescence of gossypol (see text).
[c] The emission lifetime, in s. – [d] The radiative lifetime, in s.

Table 3. Observed and calculated transition energies and oscillator strengths for the 7-O$^-$ anionic form of gossypol.

Observed		Calculated		
Energy (cm^{-1})	Molar absorption coefficient (M^{-1} cm^{-1})	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
		A	19 800	0.00004
		A	22 400	0.002
		A	24 100	0.0002
25 400	19 000	A	24 900	0.178
		A	26 900	0.0002
		A	28 400	0.050
		A	29 000	0.036
		A	29 200	0.117
		A	29 300	0.076
		A	29 400	0.105
33 600	24 000	A	30 500	0.114
		A	31 800	0.005
		A	32 300	0.035
		A	33 400	0.001
~36 500	31 000	A	34 900	1.091
		A	36 200	0.366
		A	37 100	0.001
		A	37 300	0.209
41 300	76 000	A	37 900	0.878
Triplet states				
–	–	A	19 900	0.006 [b]
		A	22 500	0.001 [b]

[a] From absorption spectra in ethanol buffered pH = 9.5.
[b] The radiative lifetime, in s.

strengths of the long-wavelength absorption band upon deprotonation.

The following conclusions can be drawn from a comparison of the experimental and theoretical results:

- The lowest excited singlet state of the gossypol molecule is calculated to be of nπ^* type. This is consistent with the spectral characteristics of this state: lack of a room temperature fluorescence and weak long-wavelength absorption (with the band observed in hexane solution at ~430 nm, $\varepsilon \sim 100$).
- The red shift of the long-wavelength absorption band III with increasing solvent polarity (see Table 1) is consistent with the calculated π-π^* character of the excited state.
- Changes in the dihedral angle between the naphthalene subunits of gossypol molecule (±30° from the crystallographic value) do not affect significantly the calculated absorption spectra.
- The absorption spectra observed at different pH values (pH = 4–10) can be attributed to those of O$^-$ anionic forms. A comparison of calculated charge densities on oxygen atoms of the OH groups in the ground state suggests the formation of the anionic form of gossypol in the position 7 (*ortho*-position to the aldehyde group). For the 7-O$^-$ anionic form of gossypol absorption bands are calculated to be shifted to longer wavelengths in comparison to the neutral molecule. That is consistent with changes observed in the experimental spectra.

2. Photochemical Stability of Gossypol in Solution

Photodecomposition of gossypol by ultraviolet light was recently studied by Abou-Donia et al. [4b]. It was shown that during irradiation with a medium

Table 4. The quantum yield of the gossypol decomposition in solution.

Solvent	$\Phi \times 10^3$ [a, b] $\lambda_{ex} = 366$ nm			$\Phi \times 10^3$ [a, c] $\lambda_{ex} = 254$ nm		
	saturated with oxygen	saturated with air	deoxygenated	saturated with oxygen	saturated with air	deoxygenated
C_2H_5OH (buffered pH = 5.2)	0.62	0.39	<0.009	–	–	–
C_2H_5OH	0.53	0.32	<0.009	1.3	0.9	0.9
C_2H_5OH (buffered pH = 9.1)	0.05	0.04	<0.009	–	–	–
CCl_4	0.52	0.35	<0.002	–	–	–
n-C_6H_{14}	0.10	0.08	<0.009	7.2	6.1	2.7

[a] Average value of at least three independent series of irradiations.
[b] Benzophenone-benzhydrol actinometer [16]. – [c] Uranyl oxalate actinometer [16].

pressure mercury lamp in quartz cells ($\lambda > 238$ nm) gossypol was decomposed, and the rate of decomposition was dependent on the solvent used. Although the degradation products were not identified, it was shown that they no longer had an aromatic structure. It was concluded that as a potential contraceptive agent, gossypol should be protected from light when used as a drug.

In this study, more quantitative data on the photochemical properties of gossypol in solution are presented, including the influence of the excitation wavelength and oxygen. The gossypol solutions were irradiated at $\lambda > 330$ nm (excitation to a low-lying excited singlet state) under nitrogen and in oxygen or air saturated solutions. The reaction was monitored by UV-visible and TLC analysis. The photodecomposition of gossypol was observed only in the presence of oxygen, and deoxygenation inhibited this photoreaction. Because of the large number of unstable photooxidation products formed with low chemical yields, our attempts to separate and identify at least some of them were unsuccessful (TLC analysis).

The quantum yields of gossypol decomposition were determined for irradiation with monochromatic light of $\lambda = 366$ nm. The values of quantum yields were significantly dependent on the solvent, pH, and concentration of oxygen (Table 4).

As can be expected, for an oxygenated basic solution a photooxidation of the anionic form of gossypol can occur, but quantum yields are much different from those for the neutral form of gossypol (Table 4). This is consistent with the results of recent studies of Tyshenko et al. [7] by means of EPR technique.

The photolysis with $\lambda = 254$ nm radiation (excitation to the higher excited states) led to decomposition of gossypopl also in carefully deoxygenated solutions (Table 4). After saturation of the solutions with oxygen or air an increase of the quantum yields was observed, suggesting that under these conditions also a photooxidation occurred.

Studies with typical singlet oxygen sensitizers (e.g. methylene blue) and quenchers (e.g. sodium azide), using the procedure described previously for the photooxidation of Y_t base [17], showed that a mechanism of photooxidation of gossypol via singlet oxygen can be excluded. The general reaction scheme for the photooxidation of gossypol (G) under the experimental conditions used in this study may be preliminarily expressed as follows:

$$\mathrm{G} \xrightarrow[(\lambda\,=\,366\ \mathrm{nm})]{h\nu} \mathrm{G^*} \xrightarrow{\mathrm{O_2}} \mathrm{GOO} \longrightarrow \text{Products}.$$

In summary, this study demonstrates that gossypol is photochemically stable in deoxygenated solutions during irradiation in glass cells ($\lambda > 330$ nm). Saturation with oxygen leads to photooxidation of gossypol. The photodecomposition of gossypol in deoxygenated solution can occur during irradiation in quartz cells with a high energy radiation ($\lambda = 254$ nm).

Acknowledgement

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