Molecular Structures and Stability Constants of Gossypol and Its Aza-Derivative Complexes with Silver(I) Cations Studied by Potentiometric, ESI MS, NMR, and AM1d Semiempirical Methods

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Received: May 16, 2008; Revised Manuscript Received: June 27, 2008

Stability constants of complexes formed by gossypol and by ten of its Schiff bases with Ag^+ cations were determined by the potentiometric method. The potentiometric and ESI MS experiments indicate the formation of AgL^+ and Ag_2L^{2+} complexes between the Schiff bases G1-G7 and Ag^+ cations as well as the formation of AgL^+, Ag_2L^{2+}, AgL_2^+ and $Ag_3L_2^{3+}$ complexes between the Schiff bases G8-G10 and Ag^+ cations. The highest stability constant was found for the AgL^+ complex of G8 Schiff base and the lowest one for the AgL^+ complex of G molecule. The ^{13}C NMR spectra of mixtures between G and $AgClO_4$ as well as G1-G10 and $AgClO_4$ indicate that the complexation of the Ag^+ cations is exclusively realized by the aldehyde-aldehyde tautomer of gossypol and by the enamine-enamine form of gossypol Schiff bases, respectively. We show that the main coordination sites for the Ag^+ metal cations are either the oxygen or the nitrogen atoms of the amine parts of the Schiff bases of gossypol. The energetically most favorable structures of the Ag^+ complexes with gossypol (G) or with the gossypol Schiff bases (G1-G10) were calculated and visualized by the AM1d method at an semiempirical level of theory.

Introduction

Gossypol is a yellow disesquiterpene found in cottonseeds. It exhibits many useful biological properties such as antiviral, anticancer or antimalarial effects.[1−6] Despite these interesting properties, this compound is responsible for the toxicity of various cotton plants and is regarded as an unwanted component in their processing.[7] The toxicity of gossypol is based on the presence of two aldehyde groups in the molecule.[8] There are mainly two methods to chemically modify the gossypol molecule to decrease its toxicity and thus expand the number of gossypol products used in agriculture or for other applications. One is blocking of the two aldehyde groups by formation of aza-derivatives due to the reactions with amines or hydrazines and the second one is the formation of complexes between gossypol and metal cations.[9−12] These interesting and promising properties of gossypol have motivated a number of scientific teams to synthesize many different gossypol aza-derivatives not only to lower their toxicity but also to create new biological properties to expand the application range of gossypol products.[13−17]

Our earlier studies indicate that gossypol and its aza-derivatives, e.g., Schiff bases or hydrazones are able to form complexes with various biologically important metal cations such as Na^+ and K^+.[18−22] It was also shown that the complexation of monovalent metal cations by the aza-derivatives,[23] especially by gossypol Schiff bases, can be realized by different tautomeric forms.[13] However, up to now, the affinity of gossypol and of its aza-derivatives toward Ag^+ cations has not been a subject of systematic studies. Earlier studies show that the ability of complexes formation with Ag^+ cations is often accompanied by an increased antimicrobial activity.[24−26] Furthermore, a quantitative determination of the stability constants of complexes between gossypol Schiff bases and Ag^+ cations of different stoichiometry might open new fields of application of these derivatives, such as the use of Ag^+ extracting agents in supramolecular chemistry or as a new materials for development ion selective electrodes sensitive to Ag^+ cations. Chemical conversion of gossypol into its Schiff bases by introducing oxaalkyl moieties, crown ethers or sulfur and nitrogen atoms lead to new group of ligands. They are still based on gossypol but are now able to form complexes with monovalent metal cations and might therefore have potentially new interesting biological properties.

In this study we report the formation of various gossypol aza-derivatives that are able to form complexes with Ag^+ cations. For the first time the stability constants of the complexes of gossypol−Ag^+ and gossypol Schiff bases−Ag^+ complexes were determined using the potentiometric method. Complex formation with Ag^+ cations was also studied by electrospray ionization mass spectrometry (ESI MS) method, whereas the structural investigation of the complexes and their visualization were performed by ^{13}C NMR as well as by semiempirical Austin 1d methods (AM1d).

Experimental Section

5-Hydroxy-5-oxa-pentylamine, 3,6-dioxadecylamine and 3,6,9-trioxadecylamine were synthesized following the procedures described in ref 27. Other amines used were commercial products of Aldrich. Gossypol was extracted from cotton seeds of *Gossypium herbaceum* following the procedure given in ref 28.

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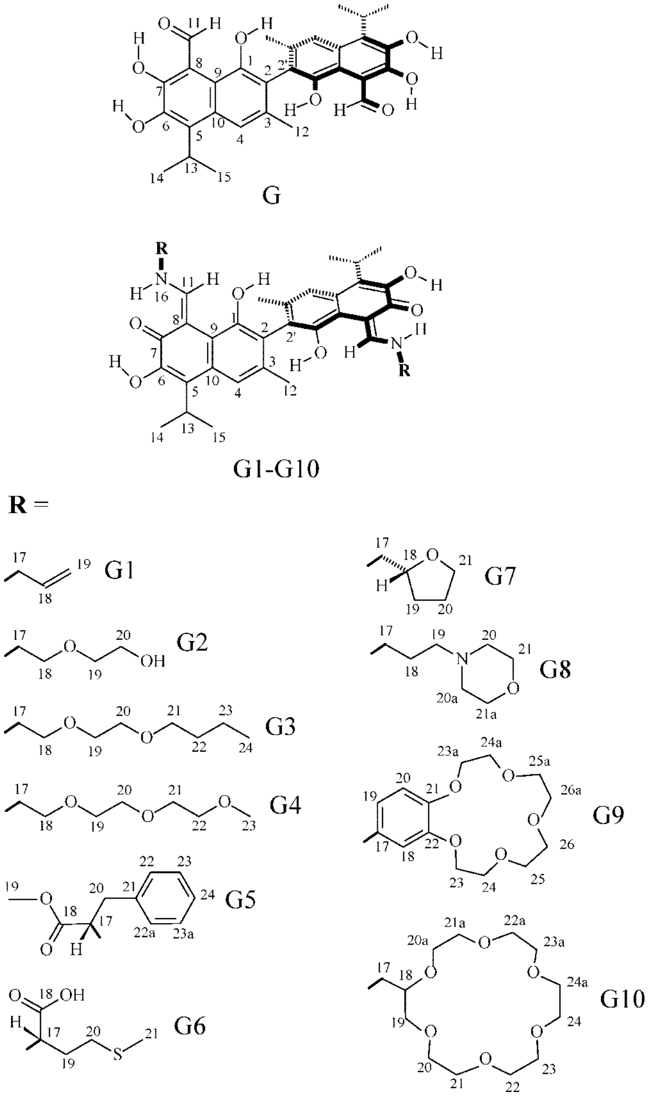
10.1021/jp8043434 CCC: $40.75

SCHEME 1: Structures and the Carbon Atom Numbering of Gossypol and Its Derivatives Studied



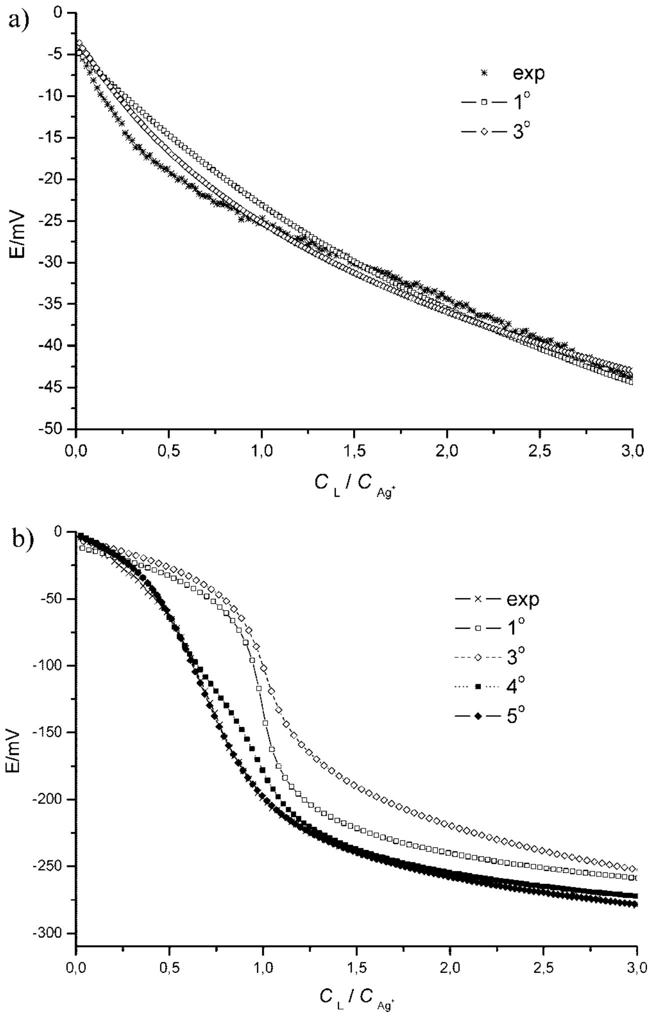


Figure 1. Potentiometric measurement of silver(I) perchlorate (6.00×10^{-3} mol L^{-1}) with (a) G3 as a function of the molar ratio C_{G3}/C_{Ag^+} [the experimental points are (*) and results of the fit by the models: 1° (□); 3° (◇)] and with (b) G10 as a function of the molar ratio C_{G10}/C_{Ag^+}[the experimental points are (×) and results of the fit by the models: 1° (□),; 3° (◇); 4° (■); 5° (◆)].

Syntheses of Schiff Bases of Gossypol. The Schiff bases of gossypol: G1-G5 and G7, G9, G10 were synthesized following our procedures described in refs 29–34, respectively. The purity of the G1−G5 and G7, G9, G10 aza-derivatives of gossypol was controlled by the ^{1}H NMR and FT-IR spectra. The synthesis, ^{1}H and ^{13}C spectroscopic characterization as well as melting points of the G6 and G8 Schiff bases are given in the Supporting Information.

Preparation of the Schiff Base Complexes with Ag^+ Cations. Because $AgClO_4$ was hydrated, it was necessary to dehydrate it by several (6−10 times) evaporation steps from a 1:5 mixture of acetonitrile and absolute ethanol. The dehydration of the silver(I) perchlorate was monitored by FT-IR spectroscopy in acetonitrile solution. The 0.05 mol L^{-1} solutions of AgL^+ and Ag_2L^{2+} complexes of G, G1−G5, and G7−G10 with Ag^+ cations were obtained by adding of the equimolar or double amounts of $AgClO_4$ (salt dissolved in absolute ethanol, 3.5×10^{-5} or 7×10^{-5} mol in 30 mL) to the Schiff base dissolved in acetonitrile (3.5×10^{-5} mol in 50 mL). The solvents were evaporated under reduced pressure to dryness. The oily residue was dissolved in dry CH_3CN (5 mL) and evaporated under reduced pressure several times to be finally dissolved in the 0.7 mL of dried CD_3CN.

ESI MS Measurements. The ESI (electrospray ionization) mass spectra were recorded on a Waters/Micromass (Manchester, U.K.) ZQ mass spectrometer equipped with a Harvard Apparatus syringe pump. All sample solutions were prepared in a mixture of chloroform with acetonitrile (1:5). The measurements were performed for two types of samples being the solutions of (a) gossypol, its Schiff bases (1×10^{-5} mol L^{-1}) with $AgClO_4$ (1×10^{-4} mol L^{-1}) used separately and (b) all Schiff bases G1−G10 with the $AgClO_4$ used as one mixture (the concentration of each Schiff base in the mixture was 1×10^{-4} mol L^{-1} and $AgClO_4$ was 1×10^{-3} mol L^{-1}). The detailed description of the ESI MS measurements is given in the Supporting Information.

Potentiometric Measurements. The potentiometric method used was described in ref 35. The potentiometric titration was performed in propylene carbonate at 25 °C using an OP-205 Radelkis pH-meter linked to a personal computer via a PCL-838 control card. The equilibrium constants were calculated using the program STOICHIO based on the nonlinear least-squares Gauss−Newton−Marquardt algorithm.[36] A detailed description of the statistical part dealing with the potentiometric measurements were deposited in the Supporting Information.

NMR Measurements. The ^{1}H and ^{13}C NMR spectra of G and G1−G10 as well as their complexes with Ag^+ cations were recorded in CD_3CN solutions (0.05 mol L^{-1}) using a Varian Gemini 300 MHz spectrometer. The ^{1}H and ^{13}C NMR spectra of

TABLE 1: Stability Constants (log β_i, log β_{2L} and log β_{32}) of the Complexes of Gossypol (G) and Its Schiff Bases (G1−G10) with Ag^+ Cations in Propylene Carbonate at 25 °C

compound	log β_1 (AgL^+)	log β_2 (Ag_2L^{2+})	log β_{2L} (AgL_2^+)	log β_{32} ($Ag_3L_2^{3+}$)
G	2.33 ± 0.18	4.75 ± 0.26		
G1	2.98 ± 0.14	6.04 ± 0.19		
G2	2.74 ± 0.17	5.57 ± 0.24		
G3	2.84 ± 0.19	5.79 ± 0.27		
G4	3.26 ± 0.08	6.94 ± 0.15		
G5	2.86 ± 0.16	6.14 ± 0.23		
G6	3.14 ± 0.09	6.66 ± 0.17		
G7	3.09 ± 0.16	6.46 ± 0.21		
G8	11.28 ± 0.05	18.13 ± 0.18	15.21 ± 0.20	33.26 ± 0.26
G9	5.06 ± 0.06	8.85 ± 0.19	7.01 ± 0.28	17.34 ± 0.34
G10	7.64 ± 0.07	13.09 ± 0.21	10.35 ± 0.23	24.53 ± 0.22

G6 and G8 were additionally recorded in DMSO-d_6 and $CDCl_3$ solutions (0.05 mol L^{-1}), respectively, using a Varian Gemini 300 MHz spectrometer. The ^1H NMR measurements of G6, G8 and complexes of G and G1−G10 compounds with Ag^+ cations were carried out at the operating frequency 300.075 MHz; flip angle, pw = 45°; spectral width, sw = 4500 Hz; acquisition time, at = 2.0 s; relaxation delay, d_1 = 1.0 s; T = 293.0 K and TMS as the internal standard. No window function or zero filing was used. Digital resolution = 0.2 Hz/point. ^{13}C NMR spectra were recorded at the operating frequency 75.454 MHz; pw = 60°; sw = 19000 Hz; at = 1.8 s; d_1 = 1.0 s; T = 293.0 K and TMS as the internal standard. Line broadening parameters were 0.5 or 1 Hz. Digital resolution = 0.2 Hz/point. The assignments of the carbon atom resonances of G and G1−G10 and their complexes in CD_3CN solution were made on the basis of HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond coherence) correlations. The errors in the ^1H and ^{13}C chemical shifts values were 0.01 and 0.1 ppm, respectively.

AM1d Calculations. AM1d calculations were performed using the Win Mopac 2007 program at the semiempirical level (Cache Work System Pro Version 7.5.085, Fujitsu).[37,38] The mode of coordination of Ag^+ cations by G and G1−G10 molecules, tautomeric forms as well as hydrogen bond pattern stabilizing the tautomeric structures, was assumed on the basis of the ^{13}C NMR data of the respective complexes as well as on the basis of crystallographic data of some gossypol Schiff bases.[32] The description of molecular modeling of G and G1−G10 complexes with Ag^+ cations is detail is given in the Supporting Information.

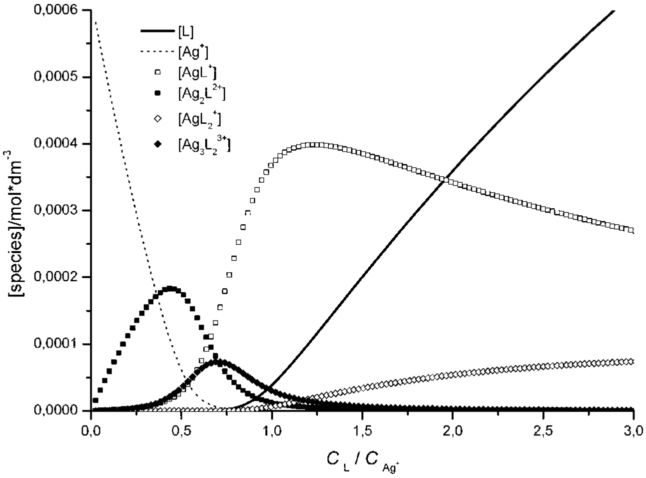


Figure 2. Exemplary diagram of distribution of AgL^+ (□), Ag_2L^{2+} (■), AgL^{2+} (◇) and $Ag_3L_2^{3+}$ (◆) complexes as a function of the molar ratio C_{G10}/C_{Ag^+} determined on the basis of the potentiometric data.

Results and Discussion

The molecular structures of gossypol (G) and its Schiff bases (G1−G10) together with the carbon atom numbering are shown in Scheme 1.

Two different types of exemplary potentiometric titration curves of Ag(I) with G3 and G10 Schiff bases of gossypol in propylene carbonate are shown in Figure 1a,b, respectively. All the curves exhibit characteristic features indicating the coexistence of different types of complexes. The models in our potentiometric investigations assume the presence of the following complexes: model 1°-AgL^+; model 2°-AgL^+, AgL_2^+; model 3°- AgL^+, Ag_2L^{2+}; model 4°- AgL^+, Ag_2L^{2+}, AgL_2^+; model 5°- AgL^+, Ag_2L^+, AgL_2^+, $Ag_3L_2^{3+}$, where L is G or G1−G10. The first type of the potentiometric titration curve (Figure 1a) is characteristic for the formation of complexes with relatively low stability constants, whereas the second one (Figure 1b) is typical of very stable complexes. The overall stability constants (β_i) of silver(I)−Schiff base complexes were calculated from the following equilibria:

$$(Ag_{(i-1)}L)^{(i-1)} + Ag^+ \xrightleftharpoons{K_i} Ag_iL^{i+} \tag{1}$$

where i = 1, 2 and L= G, G1−G10.

In particular, for the AgL_2^+ complexes between silver(I) and the Schiff bases (G8−G10) the stability constants β_{2L} were calculated from the equilibrium:

$$AgL^+ + L \xrightleftharpoons{K_{2L}} AgL_2^{\,+} \tag{2}$$

Besides the types of complexes described above, the Schiff bases (G8−G10) form complexes of the $Ag_3L_2^{3+}$ stoichiometry. The formation of the $Ag_3L_2^{3+}$ complexes is possible to achieve in various ways as it is shown for instance below:

$$Ag_2L^{2+} + AgL^+ \xrightleftharpoons{K_{32}} Ag_3L_2^{\,3+} \tag{3}$$

or

$$Ag_2L_2^{\,2+} + Ag^+ \xrightleftharpoons{K_{32}} Ag_3L_2^{3+} \tag{4}$$

or

$$AgL_2^{\,+} + 2Ag^+ \xrightleftharpoons{K_{32}} Ag_3L_2^{\,3+} \tag{5}$$

and therefore, the definition of the K_{32} stability constants is much more difficult. Because the K_{32} value is difficult to determine,

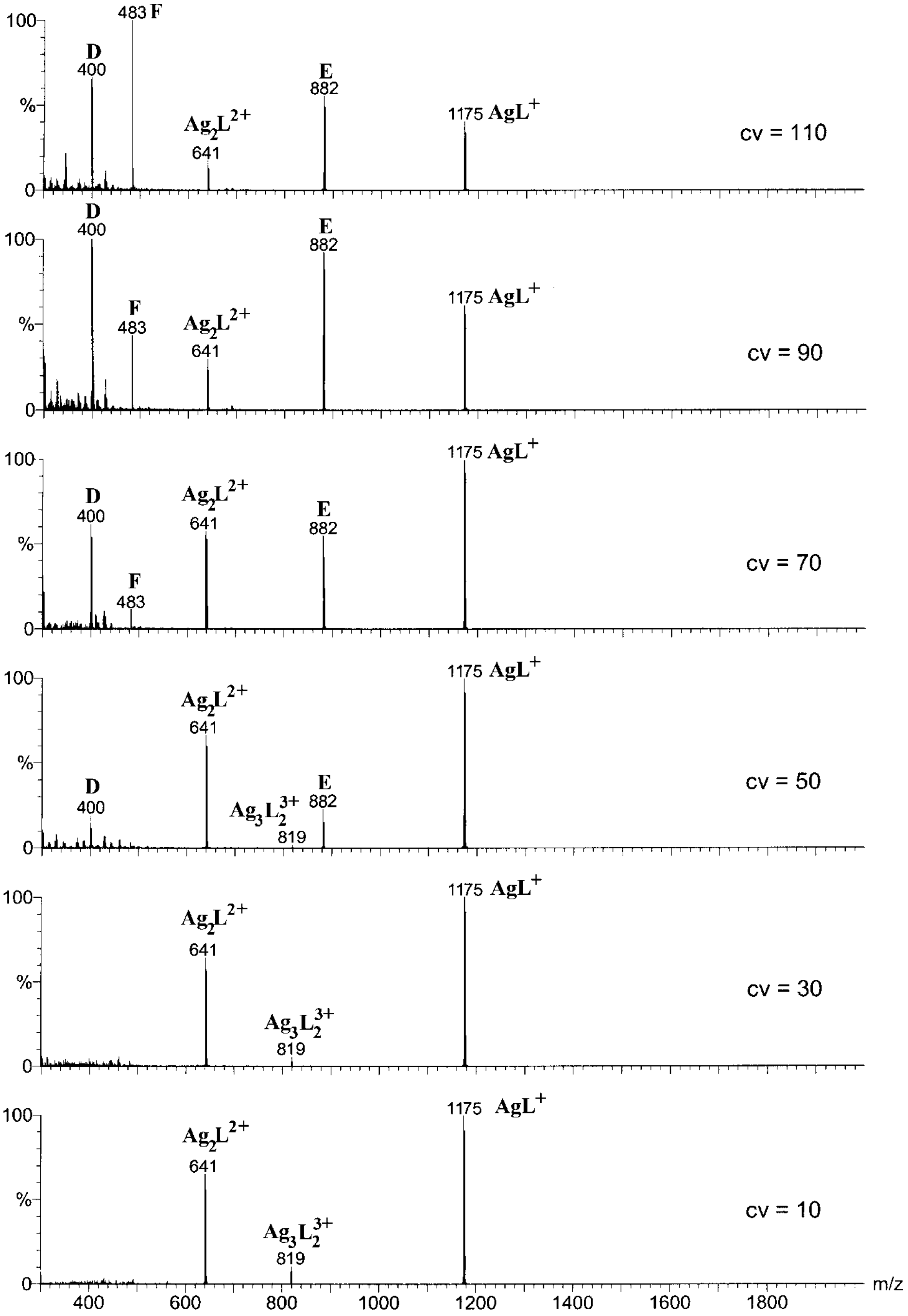


Figure 3. ESI MS spectra of the 1:10 mixture of G10 with the $AgClO_4$ at various cone voltages cv = 10, 30, 50, 70, 90 and 110 V.

we can estimate the overall stability constant β_{32} values defined as

$$3Ag^+ + 2L \overset{\beta_{32}}{\rightleftharpoons} Ag_3L_2^{\ 3+} \qquad (6)$$

The stability constants of silver(I) complexes with G and its Schiff bases in propylene carbonate at 25 °C are collected in Table 1. The results of the fit of the various equilibrium models to the experimental titration curve have shown that the equilibrium model of the coexistence of AgL^+ and Ag_2L^{2+} complexes (model 3°, Figure 1a) is adequate for G and its Schiff bases G1−G7 being relatively poor ligands toward the Ag^+ cations. In the ESI MS spectra of the mixtures of these compounds with $AgClO_4$, two *m/z* signals of AgL^+ and Ag_2L^{2+} complexes are observed indicating the presence of only two types of complexes. For the G8−G10 Schiff bases the inclusion of AgL_2^+ and $Ag_3L_2^{3+}$ complexes to the model 3° (model 5°) significantly improves the fit of the theoretical curve to the experimental one as illustrated in Figure 1b and by the fit coefficients collected in Table 1S (Supporting Information). The β values calculated for the AgL_2^+ and $Ag_3L_2^{3+}$ demonstrate that these types of complexes play an important role in the equilibria in solution. The exemplary diagram determined for G10 (Figure 2) presents the dependence of the concentration of AgL^+, Ag_2L^+, AgL_2^+, $Ag_3L_2^{3+}$ species on the molar ratio C_{G10}/C_{Ag^+}. At the ratio $C_{G10}/C_{Ag^+} < 1.0$ the main role play complexes consisting of higher number of metal cations ($Ag_3L_2^{3+}$, Ag_2L^{2+}) whereas at the ratio $C_{G10}/C_{Ag^+} > 1.0$ the contribution of the complex consisting of lower number of metal cations in the equilibria increases. Despite the relatively low value of the maximum concentration of $Ag_3L_2^{3+}$ complex on the diagram

TABLE 2: Enthalpy of Formation (kcal/mol) of Gossypol and Its Schiff Bases Complexes with Ag^+ Cations Calculated by the AM1d Method (WinMopac 2007)[a]

complex	type of the complex	ΔH_f (kcal/mol)	$\Delta H_f°/(Ag^+)_n$ (kcal/mol)
G−$Ag^{+}_{\text{uncomplexed}}$	AgL	−32.03	−32.03
G−$Ag^{+}_{\text{complexed}}$			
G1−$Ag^{+}_{\text{uncomplexed}}$		−41.83	−41.83
G1−$Ag^{+}_{\text{complexed}}$			
G2−$Ag^{+}_{\text{uncomplexed}}$		−35.17	−35.17
G2−$Ag^{+}_{\text{complexed}}$			
G3−$Ag^{+}_{\text{uncomplexed}}$		−38.94	−38.94
G3−$Ag^{+}_{\text{complexed}}$			
G4−$Ag^{+}_{\text{uncomplexed}}$		−57.91	−57.62
G4--$Ag^{+}_{\text{complexed}}$			
G5−$Ag^{+}_{\text{uncomplexed}}$		−39.02	−39.02
G5−$Ag^{+}_{\text{complexed}}$			
G6−$Ag^{+}_{\text{uncomplexed}}$		−48.27	−48.27
G6−$Ag^{+}_{\text{complexed}}$			
G7−$Ag^{+}_{\text{uncomplexed}}$		−42.19	−42.19
G7−$Ag^{+}_{\text{complexed}}$			
G8−$Ag^{+}_{\text{uncomplexed}}$		−241.85	−241.85
G8−$Ag^{+}_{\text{complexed}}$			
G9−$Ag^{+}_{\text{uncomplexed}}$		−143.56	−143.56
G9−$Ag^{+}_{\text{complexed}}$			
G10−$Ag^{+}_{\text{uncomplexed}}$		−189.35	−189.35
G10−$Ag^{+}_{\text{complexed}}$			
G−2$Ag^{+}_{\text{uncomplexed}}$	Ag_2L	−65.12	−32.56
G−2$Ag^{+}_{\text{complexed}}$			
G1−2$Ag^{+}_{\text{uncomplexed}}$		−86.77	−43.39
G1−2$Ag^{+}_{\text{complexed}}$			
G2−2$Ag^{+}_{\text{uncomplexed}}$		−71.58	−35.79
G2−2$Ag^{+}_{\text{complexed}}$			
G3−2$Ag^{+}_{\text{uncomplexed}}$		−79.64	−39.82
G3−2$Ag^{+}_{\text{complexed}}$			
G4−2$Ag^{+}_{\text{uncomplexed}}$		−120.32	−60.16
G4−2$Ag^{+}_{\text{complexed}}$			
G5−2$Ag^{+}_{\text{uncomplexed}}$		−92.24	−46.12
G5−2$Ag^{+}_{\text{complexed}}$			
G6−2$Ag^{+}_{\text{uncomplexed}}$		−116.22	−58.11
G6−2$Ag^{+}_{\text{complexed}}$			
G7−2$Ag^{+}_{\text{uncomplexed}}$		−93.73	−46.87
G7−2$Ag^{+}_{\text{complexed}}$			
G8−2$Ag^{+}_{\text{uncomplexed}}$		−162.63	−81.32
G8−2$Ag^{+}_{\text{complexed}}$			
G9−2$Ag^{+}_{\text{uncomplexed}}$		−125.86	−62.93
G9−2$Ag^{+}_{\text{complexed}}$			
G10−2$Ag^{+}_{\text{uncomplexed}}$		−153.76	−76.88
G10−2$Ag^{+}_{\text{complexed}}$			

[a] $\Delta H_f° = H_f°_{\text{complexed}} - H_f°_{\text{uncomplexed}}$, where $H_f°_{\text{complexed}}$ = metal cation and the ligand molecule are bonded to each other; $H_f°_{\text{uncomplexed}}$ = metal cation and the ligand molecule are distanced from each other by more than 30 Å, assumed tautomeric form of G1−G10 is the enamine−enamine one. $(Ag^+)_n$ is the number of Ag^+ cations complexed.

(Figure 2), of about 8×10^{-5} mol dm^{-3}, the contribution of this complex in the equilibria at the molar ratio $C_{G10}/C_{Ag^+} \approx 0.7$ is significant. From the C_{G10}/C_{Ag^+} molar ratio of about ≈0.7−1.0 the concentration of the $Ag_3L_2^{3+}$ and Ag_2L^{2+} complexes decreases and the concentration of AgL_2^+ and AgL^+ complexes increases. At the molar ratio $C_{G10}/C_{Ag^+} > 1.0$ the concentration of AgL_2^+ and AgL^+ complexes increases. At the molar ratio $C_{G10}/C_{Ag^+} > 1.0$ the concentration of $Ag_3L_2^{3+}$, Ag_2L^{2+} and AgL^+ species decreases while the participation of AgL_2^+ complex in the equilibria increases. At a higher excess of the G10 ligand in the solution, the concentration of Ag_2L^{2+} complex slightly decreases whereas the concentration of AgL_2^+ slightly increases. In the ESI spectra of the mixtures of G8−G10 with Ag^+ cations, at lower cv values, besides the *m*/*z* signals assigned to the AgL^+ and Ag_2L^{2+} complexes, also the *m*/*z* signals of low and very low abundant complexes ($Ag_3L_2^{3+}$ and AgL_2^+) are observed (Figure 3 and Tables 2S and 3S, Supporting Information). A comparison of the stability constants of the complexes (Table 1) with the relative abundances of these complexes obtained from the ESI mass spectra (Tables 2S, Supporting Information) shows that these values do not correspond very well to each other. This fact is probably concerned with the different experimental conditions applied for the potentiometric and ESI MS measurements (liquid and gas states). Thus, the detection of four types of ions: AgL^+, Ag_2L^{2+}, $Ag_3L_2^{3+}$ and AgL_2^+ confirms the model 5° assumed for G8−G10 complexes in the potentiometric investigation. In the ESI MS spectra, recorded at various cone voltage values, the presence of the fragmentation ions of G and G1-G10 complexes with Ag^+ cations was noted (Table 3S, Supporting Information). The respective fragmentation pathways of these complexes are shown in Scheme 1S of the Supporting Information.

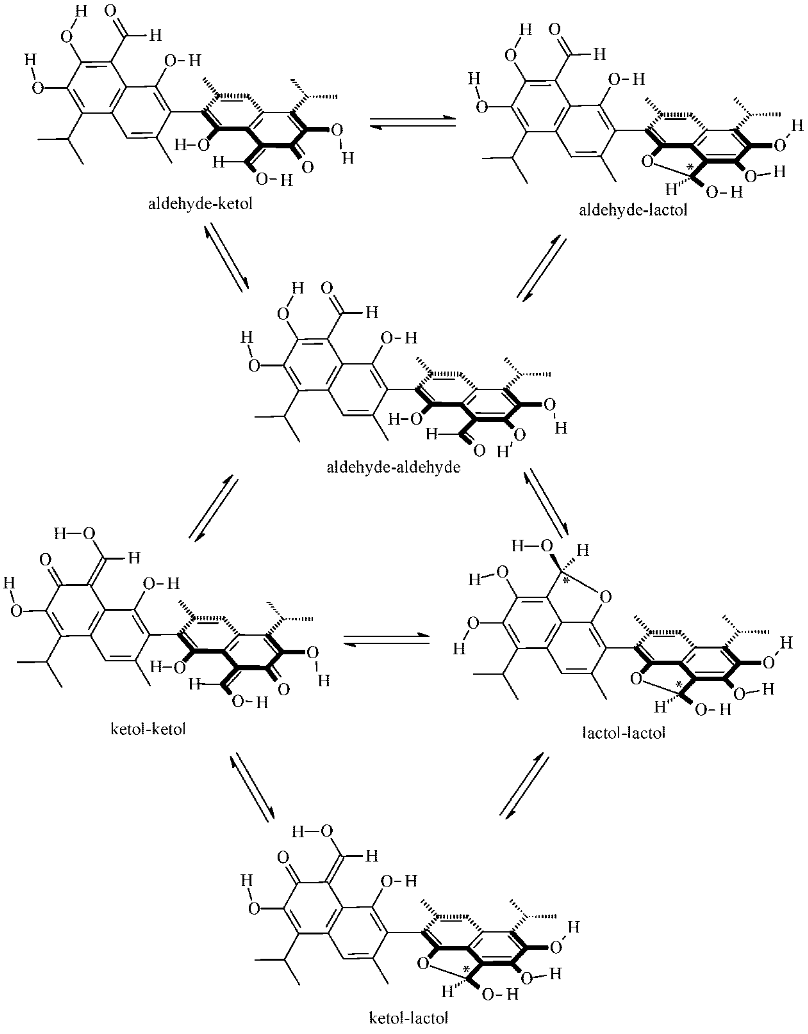
The stability constants of the AgL^+ complexes are much higher than the respective constant for the corresponding Ag_2L^{2+} complexes formed by G8−G10 (Table 1 and Figure 2). In contrast, the stability constants of G and its Schiff base G1−G7 complexes with silver(I) cations indicate a preferential formation of the Ag_2L^{2+} complexes compared to the AgL^+ complexes, although the concentration of the latter ones in the equilibria is higher. It is interesting to note that the stability constant of the AgL^+ complex with the G8 Schiff base is comparable with other ones described in the literature and determined for selective ligands toward Ag^+ cations.[39−45] The lowest stability constant of all the complexes with Ag^+ cations that were studied was found for the G complexes. For this reason in the ESI spectrum of a mixture of G with Ag^+ cations no *m*/*z* signals of the respective complexes are observed, indicating that the unmodified gossypol molecule, as it occurs in cotton plants, is the weakest ligands under investigation (Table 3S, Supporting Information).

The structures of the respective G and G1−G10 complexes with Ag^+ cations were investigated by ^{13}C NMR and visualized by the AM1d semiempirical method. The ^{13}C NMR data of gossypol and amine parts of G, G1−G7 and G8−G10 are deposited as Supporting Information in Tables 4S−7S, respectively, whereas the values of enthalpy of formation ($\Delta H_f°$) of the most energetically favorable AgL^+ and Ag_2L^{2+} complexes of G1-G10 with Ag^+ cations are collected in Table 2.

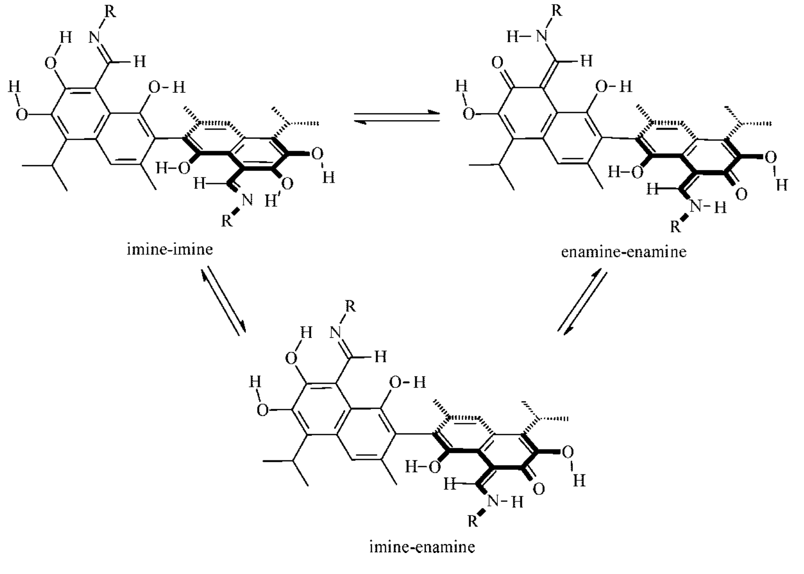
In solution gossypol can be present in various tautomeric forms (Scheme 2). From earlier studies it is known that in chloroform and acetonitrile solution the aldehyde−aldehyde tautomeric form is the predominant one.[13,46] In the ^{13}C NMR spectrum of G in acetonitrile solution the most deshielded and intense signal of the C-11 carbon atom of the aldehyde group is found at 201.4 ppm, indicating a high concentration of the aldehyde−aldehyde tautomeric form of G. The presence of this tautomeric form is further confirmed by the appearance of the signal of C-7 phenolic carbon atom at 151.4 ppm. The existence of the lactol−lactol tautomer in solution is evidenced by the low intense signal of the C-11 carbon atom at 99.2 ppm. The chemical shift of the C-11 carbon atom of the lactol−lactol tautomer is found in the range typical of other compounds with the lactol moiety.[47] In the ^{13}C NMR spectrum of the 1:2 complex of G:Ag^+, the greatest chemical shift changes were noted for the C-11 ($\Delta\delta = -0.8$ ppm) and C-7 ($\Delta\delta = +0.7$ ppm) carbon atom signals. Furthermore, in this spectrum the C-11 carbon atom signal of the lactol−lactol tautomer, usually present at about 99 ppm, is no longer observed. These observations demonstrate a change in the tautomeric equilibrium toward an exclusive formation of the aldehyde−aldehyde tautomer and a simultaneous coordina-

SCHEME 2: Structures of Possible Tautomeric Forms of Gossypol and Its Aza-Derivatives

GOSSYPOL



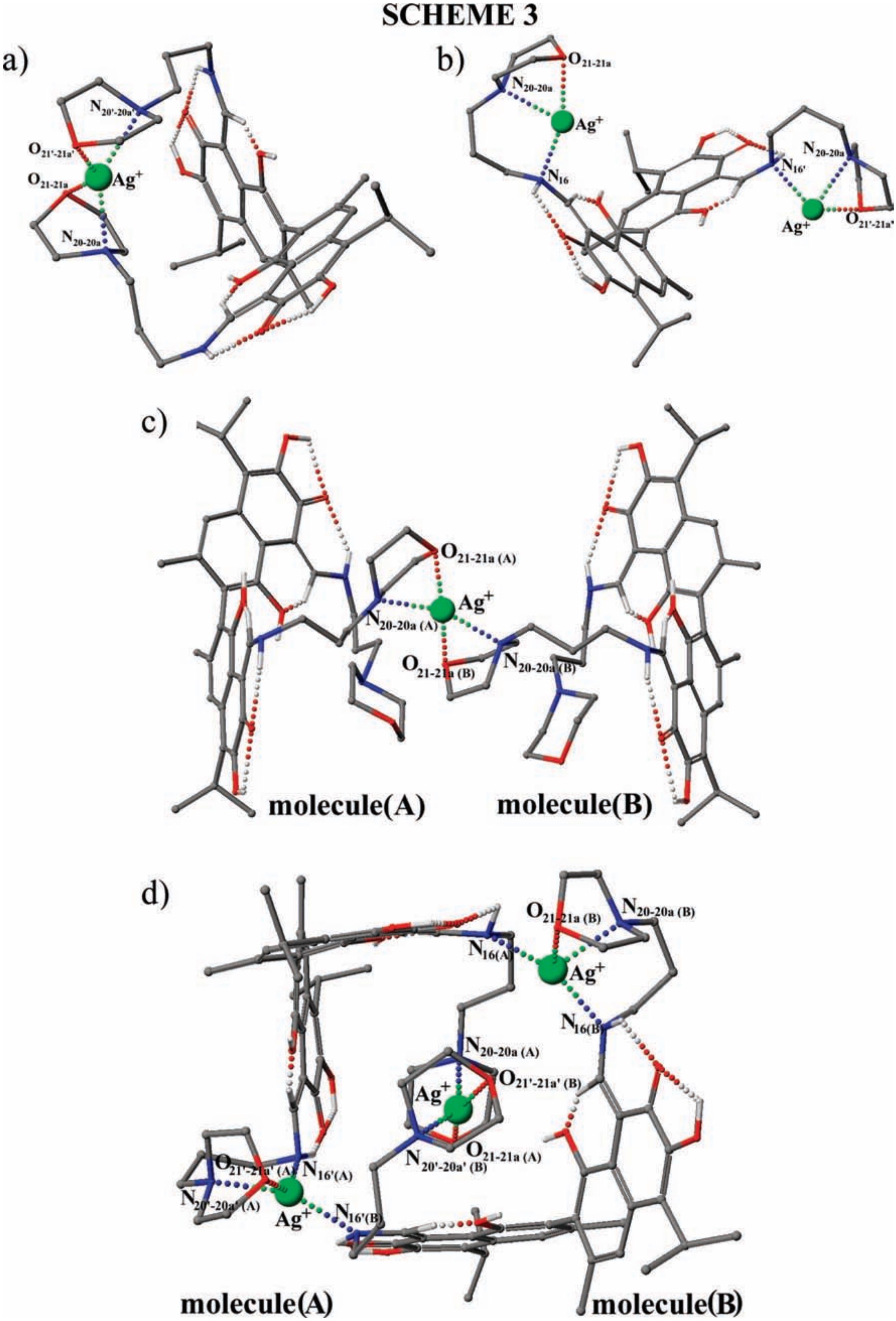
SCHIFF BASES OF GOSSYPOL



tion of the two Ag^+ metal cations by the aldehyde and O(7)H groups within both parts of G molecule as it is also indicated by the AM1d calculations (Scheme 2S, Supporting Information).

It is known that in the ^{13}C NMR spectra of the enamine−enamine tautomeric forms of Schiff bases of gossypol, the values of the C-7 and C-11 chemical shifts are of about 172 and 163 ppm,

SCHEME 3: Most Favourable Structures of the G8 Complexes with Ag^+ Cations: (a) AgL^+, (b) Ag_2L^{2+}, (c) AgL_2^+, (d) $Ag_3L_2^{3+}$, Calculated by the AM1d Method (Cache WS. Pro 7.5, Win Mopac 2007)



respectively.[48] In ^{13}C NMR spectra of G1−G10 the signals of C-7 and C-11 are also observed at similar ppm values, indicating that these Schiff bases exist in CD_3CN solutions in the enamine−enamine tautomeric forms. For the G6 and G8 compounds, ^{13}C NMR spectra in the DMSO-d_6 and $CDCl_3$ solvents, respectively, were additionally recorded (Supporting Information). In these spectra the values of the C-7 resonances were found at about 172 ppm, as in the respective spectra in

CD_3CN, which is confirmation of the presence of the enamine-enamine tautomer in G6 and G8 Schiff bases. A comparison of the ^{13}C NMR data of G1−G10 and their 1:1 and 1:2 complexes with Ag^+ cations (Tables 4S−7S, Supporting Information) shows that after the complexation of Ag^+ cations the C-7 and C-11 carbon atom signals remain in the range characteristic of the enamine-enamine tautomers. Thus, the changes of the chemical shift of these two C-7 and C-11 carbon atom signals, observed in the spectra of 1:1 and 1:2 complexes of G1−G10 Schiff bases with Ag^+ cations, are related to the complexation process. This becomes obvious from the spectra of G1, G6 and G7 Schiff base complexes, because the signal of the C-7 carbon atom of the carbonyl group is shifted toward lower ppm values indicating that the oxygen atom of this group participates in the complexation process. In the spectra of G2−G5, G8−G10 complexes with Ag^+ cations, changes in the chemical shifts are only observed to a limited extent, demonstrating that in these structures the oxygen atom of the C-7 carbonyl group is not involved in the coordination process. It is interesting to note that in the spectra of the 1:1 complexes, the C-7 resonance arises as two noncoupled signals due to the involvement of only one part of the symmetrical Schiff base molecule in the coordination process. In the ^{13}C NMR spectra of 1:2 complexes the C-7 resonance is one signal indicating the involvement of both parts of the Schiff base molecule in the complexation of Ag^+ cation.

The comparison of the ^{13}C resonances assigned to the carbon atoms of the amine parts of G1−G10 and its complexes clearly demonstrates that in complexes of 1:1 and 1:2 stoichiometry some oxygen atoms of these parts are involved in the coordination of Ag^+ cations. It should be pointed out that the greatest changes in the chemical shifts of the carbon atom signals of the amine part after complexation were found for the G8 Schiff base whose complexes with Ag^+ cations exhibit the highest stability constants.

The values of enthalpy of formation ($\Delta H_f°$) of the most favorable AgL^+ and Ag_2L^{2+} complexes of G1−G10 with Ag^+ cations, in which the Schiff base is present in the enamine-enamine tautomeric form, are collected in Table 2. The $\Delta H_f°/(Ag^+)_n$ values for AgL^+ and Ag_2L^{2+} complexes with G1−G7 Schiff bases are comparable, whereas for G8−G10 Schiff bases these values are significantly higher for AgL^+ than for Ag_2L^{2+} complexes. The most energetically favorable complex is that of AgL^+ type formed between G8 and Ag^+ cation whereas the worst ligand of Ag^+ complexation is the G molecule. This result is consistent with the stability constants determined. The decreasing partial charges of the metal cations in the complex structures relative to the charge of metal cation before complexation indicate that these cations are involved in the coordination processes (Table 8S, Supporting Information).

The structures of the most favorable G8 complexes with Ag^+ cations, which exist in the enamine−enamine tautomeric form (AgL^+, Ag_2L^{2+} and AgL_2^+ and $Ag_3L_2^{3+}$) are given in Scheme 3a−d, respectively. The highest stability of the G8:Ag^+ complex (Scheme 3a) can result from the nearly tetrahedral coordination sphere of the silver cation, formed by the two nitrogen and two oxygen atoms of the morpholine rings, localized one above the other and mutually rotated by about 90 degrees. The structures of the other complexes of G1−G7, G9 and G10 Schiff bases with Ag^+ were calculated and visualized in (Schemes 2S−4S, Supporting Information). These calculated structures show that the O_6 and O_1 atoms of the hydroxyl groups of the gossypol parts are involved in the intramolecular hydrogen bonds and play no role in the coordination process of the Ag^+ cations, whereas the new groups that were introduced to the gossypol molecule play a major role in the coordination of Ag^+ cations. This result is consistent with the NMR spectroscopic data.

In summary we can conclude that the highest energetic profits of Ag^+ complexation by G8−G10 can be explained by the high number of atoms that coordinate the metal cation (from four to six) and by the formation of almost regular octahedral or tetrahedral coordination spheres for the Ag^+ cation in the structures of the complexes studied. The relatively high stability of the Ag_2L^{2+} complexes formed between gossypol aza-derivatives containing crown-ethers (G7 and G8) and Ag^+ cations is concerned with the well-known macrocycle effect.[49−51] In the structure of G1−G7 complexes the coordination of the Ag^+ cations is realized mainly by three (in the G4 complex) donor atoms and the coordination sphere of the metal cation is irregular, allowing for the interaction between the metal cation and the solvent; therefore the stability of these complexes is lower. The importance of the coordination sphere geometry becomes apparent in the case of G6−Ag^+ complexes. Despite the fact that G6 molecule contains two sulfur donor atoms, which excellently can coordinate silver(I) cations, the stability of G6−Ag^+ complexes is relatively low because of the formation of irregular coordination sphere of the Ag^+ cation within these complexes (Scheme 2Sg, Supporting Information).

Conclusion

Ten gossypol Schiff bases have been synthesized to improve complex formation with Ag^+ cations. The complexation of Ag^+ cations has been studied using the potentiometric, ESI MS, ^{13}C NMR and AM1d methods. The stability constants of the complexes between gossypol and Ag^+ as well as between gossypol Schiff bases and Ag^+ cations were determined using the potentiometric method. The potentiometric and the ESI MS data indicate the formation of AgL^+ and Ag_2L^{2+} complexes between G1−G7 Schiff bases and Ag^+ cations as well as the formation of AgL^+, Ag_2L^{2+}, AgL_2^+ and $Ag_3L_2^{3+}$ ones between G8−G10 and Ag^+ cations. The highest stability constant was found for the AgL^+ complex of G8 Schiff base and the lowest for the G molecule. The ^{13}C NMR spectra of the gossypol Schiff base−Ag^+ complexes indicate the presence of the enamine−enamine tautomeric form within the complexes. Analysis of these spectra showed that the complexation of Ag^+ cations is mainly realized by the oxygen and the nitrogen atoms of the amine part of the Schiff base. The most energetically favorable structures of the gossypol complexes and of gossypol ten aza-derivative complexes were calculated and visualized by the AM1d method. The semiempirical calculations demonstrate that in the G8−Ag^+ complex, which has the highest stability constant, the metal cation is the most favorably coordinated in almost tetrahedral arrangement by two nitrogens and two oxygen atoms of the morpholine rings. The spectroscopic and semiempirical results indicate that only the O_7 oxygen atom of the gossypol part of the Schiff base contributes to the complexation of the Ag^+ metal cation.

Acknowledgment. Financial assistance of the Polish Ministry of Science and Higher Education, Grant No. N204 056 32/1432, is gratefully acknowledged by P.P. We thank the Polish Ministry of Science and Higher Education for financial support Grant No. R015 016 01.

Supporting Information Available: Detailed description of ESI MS, potentiometric and AM1d experimental conditions applied for investigation of silver(I) complexes with G, G1-G10. Detailed description of the method of synthesis as well as the spectroscopic data including ^{1}H and ^{13}C NMR of gossypol aza-derivatives studied. Comparison of stability constants of G3 and G10 complexes with their expected and obtained peak relative abundances [%] from ESI MS spectra at cv=10V. Results of the fit of various equilibrium models for compound G10 to potentiometric data. The NMR data of gossypol aza-derivative complexes with Ag$^+$ cations. The ESI MS fragmentation pathways of the complexes formed between gossypol derivatives and Ag(I) cations. The most favorable structures of the four types of silver(I) complexes with G, G1-G10 compounds presented in color. The selected structural parameters of the most favorable AgL$^+$ and Ag$_2$L^{2+} complexes of gossypol and its aza-derivatives with Ag$^+$ cations calculated by the AM1d method. This material is available free of charge via the Internet at http://pubs.acs.org.

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JP8043434