^1H AND ^{13}C NMR SPECTRA OF TRANSFORMATION PRODUCTS OF GOSSYPOL IN SOLUTIONS

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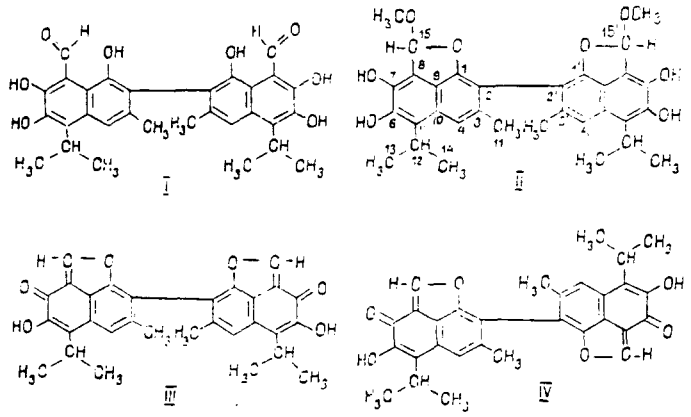
The complete cycle of the transformation of gossypol in solutions takes place in the measuring ampul of a NMR spectrometer. It has been established for the first time that, in methanol, gossypol is converted into stereoisomeric dilactol 15,15'-dimethyl ethers which change into dianhydrogossypol in chloroform and back into gossypol in aqueous acetone. The ^1H and ^{13}C NMR spectra of the main and intermediate products of the conversion of gossypol in the solvents mentioned have been studied in detail for the first time and a complete assignment of their resonance characteristics has been made. It has been shown that dianhydrogossypol is formed through intermediate stereoisomeric 15,15'-dimethyl ethers of the dilactol form of gossypol.

One of the main sources of the many-sided nature of the manifestation of chemical and physiological properties of gossypol (GP) is the capacity of this compound for existing in tautomeric forms in solutions. The properties and nature of the solvent must be taken into consideration as the decisive factor, but up to now specialists have formulated no single and clear idea about their interconnection.

There are statements in the literature on the instability of the main aldehydic form of the existence of the GP molecule in dimethyl sulfoxide [1, 2], in acid and alkaline media [3], and in alcoholic solutions [4].

Previously [5, 6], on the basis of the results of a mass spectral analysis of the products of the transformation in methanol (presence of peaks of molecular ions with M^+ 500 and M^+ 482), UV spectrometry (fall in the extinction coefficient of absorption at 376 nm due to a decrease in the amount of aldehyde groups) and literature information on the possible dehydration of GP in alcohol [7], the authors came to the conclusion that these products in low-molecular-mass alcohols are anhydro derivatives of gossypol.



We have now investigated the dynamics of the transformations of gossypol in solutions by NMR spectroscopy on ^1H and ^{13}C NMR nuclei.

When the ^1H NMR spectrum was taken, it was found that passage of GP from the dialdehyde form (I) into another state began practically from the first few minutes of the dissolution

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TABLE 1. Chemical Shifts (δ, ppm, 0 – TMS) of the Resonance Lines of Protons in the ^1H NMR Spectra of Gossypol (I), Its 15,15'-Dimethyl Ether (II), and Dianhydrogossypol (IV) in Solutions

Protons	I (CD_3OD)	II (CD_3OD)	IV ($CDCl_3$)	I ($CDCl_3$)
H-4	7,76	7,31; 7,33	7,76	7,79
H-11	2,08	2,17; 2,19; 2,23; 2,24	2,35	2,16
H-12	4,03	3,80	3,61	3,91
H-13*	1,54	1,50	1,53	1,54
H-14*	1,55	1,50	1,53	1,55
H-15	11,30	6,77; 6,79; 6,85	8,49	11,15
HO-1	—	—	—	15,22
HO-6	—	—	7,40	6,44
HO-7	—	—	—	5,78
H_3CO-15	—	3,33; 3,41	—	—

*The H-13 and H-14 signals appeared in the form of a doublet with ^3J = 7.1-7.2 Hz, and that of H-12 in the form of a complex multiplet. The other resonance lines had the form of singlets or broadened singlets. The ^1H NMR spectra were taken on WM-400 spectrometer (Bruker).

of the sample in deuterated ethanol, directly in the measuring ampul of the spectrometer. At 27°C the process under consideration took place slowly, on the whole (more than 10 days) and then gradually stabilized. The final phase of stabilization proceeded at an even slower rate. This solution, after complete stabilization had been achieved, was used to record the ^{13}C NMR spectra.

The ^1H NMR spectrum of a freshly prepared solution of GP in CD_3OD (stage A) was characterized by the following resonance lines: doublets at 1.54 and 1.55 ppm with ^3J = 7.1 Hz – the protons of the methyls of isopropyl groups; 2.08 ppm, broadened singlet – CH_3-11*; 4.03 ppm, multiplet – H-12; 7.76 ppm, broadened singlet – H-4; and 11.30 ppm, singlet – aldehydic H-15. With the exception of the absence of the signals of the hydroxy groups in view of the exchange OH ⇄ OD and the chemical shift of H-12, the spectra of solutions of GP in CD_3OD and $CDCl_3$ were practically identical.

Then the general pattern of the spectrum began to change appreciably. After the first hour from the moment of the solution of GP in CD_3OD the intensity of the signals mentioned above decreased and together with them other signals appeared similar in form but substantially differing in chemical shift, the intensity of which gradually increased. What has been said does not relate to the signal of the aldehydic proton, instead of which resonance lines appeared in another region of the spectrum – at 6.77, 6.79, and 6.85 ppm (Table 1).

In stage B of the transformation of the spectrum under investigation, when the ratio of the total integral intensities of the initial and the newly formed signals amounted to 1:1, the spectrum acquired its most complex pattern. For example, for H-4 it was possible to detect four clearly separated signals at 7.32, 7.42, 7.68, and 7.76 ppm, and there were individual resonance lines of different intensities at 2.08, 2.10, 2.15, 2.19, and 2.24 ppm from the CH_3–11 protons and four singlets at 6.77, 6.79, 6.83, and 6.84 ppm. Then the changes in the spectrum amounted to a decrease in the intensities of a number of signals and the formation of a main group of resonance lines reflecting the final stage C of the transformation being described. At the end of the second week of observations the spectrum had practically ceased to change and the general pattern stabilized.

In the final ^1H NMR spectrum of the transformation products of GP in CD_3OD a generalized doublet at 1.50 ppm with ^3J = 7.2 Hz corresponded to the protons of the methyls of the isopropyl groups. Instead of the single broadened singlet at 2.08 ppm in the initial spectrum due to CH_3–11, four signals appeared at 2.17, 2.19, 2.23, and 2.24 the total integral intensity of which was equal to half that for CH_3-13 and CH_3-14. Two broadened signals of

*The numbering of the atoms and groups of only one half of the gossypol molecule is given: the contributions of the atoms and groups of the other half with the corresponding primed numbers must apparently be understood – translator.

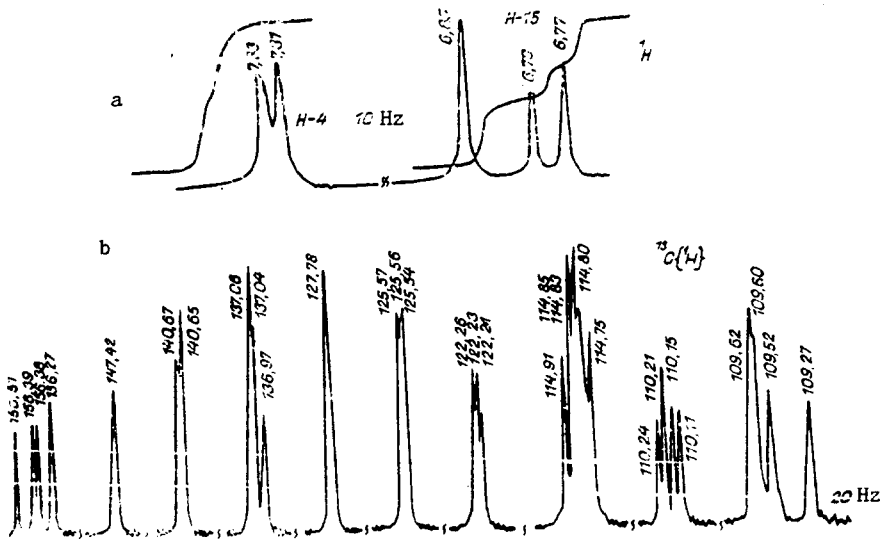

Fig. 1. Parts of the ^1H (a) and $^{13}C\{^1H\}$ (b) NMR spectra of the 15,15'-dimethyl ether of the dilactol form of gossypol (II).

approximately the same intensity were located at 7.31 and 7.33 ppm, and when an additional radiofrequency field was applied to the protons responsible for them in the double-resonance regime a contraction of the lines from the signals due to the CH_3-11 proton was observed. Consequently, these two signals related to the aromatic protons of the naphthyl nuclei, H-4. The total integral intensities of another group of singlets at 6.77, 6.79, and 6.85 ppm and of the two H-4 resonance lines were equal to one another and, therefore, the former belonged to the H-15 protons (Fig. 1, a).

Analysis of characteristics of the ^1H NMR spectra known from the literature and a comparison of them with our results for the transformation products of gossypol in CD_3OD enabled the pattern of connection of the values of the H-15 chemical shifts to be generalized and permitted them to be linked with the forms of GP and its derivatives that exist in solution. These results are collected in Table 2. From this it can clearly be seen that the signals of the H-15 protons undergo the greatest diamagnetic shift in the spectra of the hexamethyl ethers of gossypol [1] and, in our experiments, in the products of the transformation of GP in methanol, and the values of their chemical shifts are practically identical with one another: 6.82-6.96 and 6.77-6.85 ppm, respectively. On the basis of the simple relationship observed, we may conclude that the product of the transformation of GP in methanol which is under consideration also exists in the dilactol form and is the ether of the intramolecular 15,1-semiacetal of gossypol. This is shown by the characteristic diamagnetic shift of the H-4 signal by 0.4 ppm on the appearance of the dilactol forms of compounds II.

To establish unambiguously the fact that compound (II) was a dimethyl ether, we also investigated the ^1H NMR spectrum of the product of transformation of GP not in CD_3OD but in CH_3OH. For this, a solution of the product under investigation in CH_3OH previously frozen in liquid nitrogen was dried in vacuum and the dry residue was dissolved in CD_3OD. In the spectrum of this freshly prepared solution two broadened singlets corresponding to the OCH_3 groups of the compound (II) under investigation were observed at 3.33 and 3.41 ppm (the second was superposed on the multiplet from the other protons of the methyl group of the solvent). From the values of the chemical shifts, these signals are characteristic for O-methyl ethers at C-15 [1]. In the course of time, the intensities of these two singlets gradually fell and at room temperature after two days they became difficult to observe, while the general spectrum became identical with that of product (II) obtained in CD_3OD.

Thus, on the basis of all that has been said above it may be concluded that in methanol (I) is converted not into anhydrogossypol as was reported previously [5] but into 15,15'-dimethyl ethers of stereoisomeric dilactol forms of gossypol (II). This is the first time that such a gossypol derivative has been obtained. Furthermore, it has been established that the di-O-methyl groups are mobile, and in CD_3OD they change into $-OCD_3$ groups, and this change takes place by the reversible scheme

TABLE 2. Ranges of the Values of the Chemical Shifts of the H-15 Protons as Functions of the Form of Existence of GP and Its Derivatives in Solutions

Form of GP	δ, ppm, H-15
Dialdehyde form – GP and its methyl ethers	10,50 – 11.30 [3]
Ketoamine form – ketoamines of GP	10,00 – 10,40 [8,9]
Phenolimine form – arylimines of GP	9,40 – 9,80 [8,9]
Dianhydrogossypol (in acetone)	8,74 [2]
Dianhydrogossypol – compound (IV) in the present paper (in $CDCl_3$)	8,49
Dilactol form of GP – as the hexamethyl ether	6.82-6.96 [1]
Products of the transformation of GP in CD_3OD in our case.	6,77 – 6,85

$$>HC(15)-OCH_3 \underset{CH_3OH}{\overset{CD_3OD}{\rightleftharpoons}} >HC(15)-OCD_3.$$

In principle, when compound (II) is available, by using this exchange mechanism it is possible to obtain a series of its derivatives with >HC(15)–OR functions.

Additional experimental results confirming these conclusions were obtained in an investigation of carbon magnetic resonance spectra. A solution of the products of the transformation of GP in CD_3OD in the stabilized stage C was used to record two types of ^{13}C spectra – with complete suppression of spin-spin interaction with protons and without decoupling from them.

Parts of the spectrum with the complete suppression of spin-spin interaction with protons are shown in Fig. 1b, and the characteristics of the signals are given in Table 3 completely for compound (II).

Signals in the strong-field part of the spectrum at 21.34, 21.38, 21.54, and 28.06 ppm were assigned, respectively, to C-13, C-14, C-11, and C-12. In the most informative part of the spectrum, between 109.0 and 157.0 ppm, attention is directed to the following factor. In place of the expected eleven resonance lines for the individual product of the transformation of GP we counted 30 signals, these being distributed in groups, each of which contained from one to four lines with the exception of the mutually superposed five signals at 114.75-114.91 ppm from two types of carbon atoms. A comparison of the apparently single broadened signals with the actual single signals permits the assumption that the former were also formed by the superposition of four signals with close chemical shifts from monotypical carbon atoms of related molecules.

In confirmation of this it is possible to give the following experimental facts. In the high-resolution ^{13}C NMR spectrum of (II) we observed that each of the four signals from the strong field group at 109.27, 109.52, 109.60, and 109.62 ppm was split into a doublet with $^1J_{C-H}$ = 176.9 Hz. In the weakest field at 156.27, 156.38, 156.39, and 156.51 ppm the signals were split into a doublet of doublets with $^nJ_{C-H}$ = 1.2 and 3.2 Hz. Furthermore, on the basis of these facts the two groups of signals mentioned can be assigned to C-15 and C-7, respectively. Among the groups of signals between 114.75 and 114.91 ppm a single broadened singlet at 114.80 ppm appeared in the high-resolution spectrum in the form of a doublet with broadened components having $^1J_{C-H}$ = 157.4 Hz and it therefore corresponds to C-4 of the naphthyl nuclei. The other signals of this group retained their singlet nature.

The facts given above permit the conclusion that the ^{13}C NMR spectrum of (II) under consideration is a summary one. Below, let us consider how its formation is explained and by what principle four signals are observed in each of the groups of resonance lines with clear resolution.

It is known that the formation of a lactol ring leads to the appearance of an asymmetric center [11]. In the present case, when it is a question of dilactol derivatives of GP, in view of the appearance of two asymmetric centers at the tertiary carbon atom, C-15 and C-15' and the presence of atropoisomerism relative to the C-2–C-2' bond [12, 3] the formation of

TABLE 3. Chemical Shifts (δ, ppm, 0 – TMS) of the Carbon Atoms in ^{13}C NMR Spectra of the Stereoisomeric Forms of the 15,15'-Dimethyl Ether of Gossypol Dilactol (II, CD_3OD, CH_3OH), Gossypol (I, $CDCl_3$), Dianhydrogossypol (IV, $CDCl_3$), and Monoanhydrogossypol ($CDCl_3$); $^nJ_{Cx-Hm}$ Values for Dian-Hydrogossypol (IV)

Atom C	Stereoisomeric forms of the 15,-15'-dimethyl ether of gossypol dilactol (II)	I [10]	Dianhydrogossypol IV, $^nJ_{Cx-Hm}$, Hz	Monoanhydro-GP		GP
				anhydro part	aldehyde part	
1	140,65; 140,67	149,9	151,36, $^3J_{-H^{15}} = 8,7$	151,93	149,66	150,44
2	122,21; 122,23; 122,26	115,4	117,46, $^3J_{-H^4} = 7,5$; $^3J_{-H^{11}} = 4,9$	118,11	116,64	116,93
3	136,97; 137,04; 137,06	133,1	136,31, $^2J_{-H^4} \simeq 1,1$; $^2J_{-H^{11}} = 5,6$	137,54	132,98	134,18
4	114,80	117,6	123,72, $^1J = 158,1$; $^3J_{-H^{11}} = 5,6$	124,09	117,9	117,9
5	127,78	133,6	130,83, $^2J_{-H^{12}} \simeq 2$; $^3J_{-H^4} \simeq 3$;	130,75	133,87	131,28
6	147,42	142,9	149,78, $^3J_{-H^{12}} = 5,6$	149,46	143,11	143,15
7	156,27; 156,38; 156,39; 156,51	155,6	175,33, $^nJ_{-Hm} < 1$	174,89	155,97	156,04
8	114,75; 114,83; 114,85; 114,91	111,2	116,64, $^2J_{-H^{15}} = 11,8$	116,42	111,61	111,91
9	110,11; 110,15; 110,21; 110,24	114,1	118,97, $^3J_{-H^4} = 8,0$; $^3J_{-H^{15}} = 4,9$	119,42	114,74	114,87
10	125,54; 125,56; 125,57	129,2	126,31, $^3J_{-H^{12}} = 5,6$	126,58	129,19	129,53
11	21,54	27,4	20,36, $^1J = 127,6$			
12	28,06	27,4	27,06, $^1J = 127,6$			
13	21,38	19,8	20,41, $^1J = 127,6$			
14	21,34	19,8	20,41, $^1J = 127,6$			
15	109,27; 109,52; 109,60; 109,62	198,2	150,26, $^1J = 208; 8$; $^5J_{-H^4} = 1,0$	150,52	198,98	199,48
16	54,14; 54,60; 54,69; 54,75					

*The ^{13}C NMR spectra were taken on a WM-400 spectrometer (Bruker).

six isomeric forms is possible. Four of them, with the same configurations of the asymmetric centers, are characterized by symmetry of the mutual spatial arrangement of the groupings in the two naphthyl moieties. However, the spatial and electronic environment of each naphthyl half in the two hybrid atropoisomeric forms with the R- and S-configurations of the C-15 and C-15' centers are identical with those of the symmetrical stereoisomers. This means that in the ^1H and ^{13}C NMR spectra investigated here groups of resonance lines should be present, each of which contains not more than four individual signals although in principle they may correspond to similar nuclei in the molecules of the six isomeric dilactol forms of GP. It is just this pattern that we actually observed in the ^{13}C NMR spectra under consideration.

The differences between the chemical shifts within each of the groups of resonance lines are not the same, and for carbon atoms they range between 0 and 0.35 ppm (see Table 3). This difference is due to the fact that the change in the configuration of the asymmetric center

is most appreciable for the chiral carbon atom, C-15, itself (0.35 ppm). Then, in decreasing order, come the figures for the neighboring and closely located nuclei: C-7 (0.19), C-8 (0.16), and C-9 (0.10 ppm). For atoms more remote from the chiral center the magnitude of this difference becomes less significant. As a result, this leads to the situation that some groups of resonance lines are represented by four signals – for example, at 110.11-110.24 ppm or 156.27-156.51 pm – and others by three and two signals – at 122.21-122.26 and 140.65-140.67 ppm, respectively. Single lines at 114.80, 127.78, or 147.42 ppm are also a consequence of the coalescence of signals of similar carbon atoms of the isomeric forms of lactones of GP with close chemical shifts. Consequently, in view of the factors discussed above in the ^{13}C NMR spectrum of a solution of the stereoisomeric dilactol forms of gossypol in methanol we observed 30 resonance lines in place of the 44 from the corresponding carbon atoms of the naphthyl nuclei and the lactol rings.

In the high-resolution ^{13}C NMR spectrum of compound (II), because of the mutual superposition of multiplets, some of the signals with close chemical shifts formed a complex pattern and their fine structure could not be interpreted. The other resonance lines are characterized by the following parameters. The carbon atoms of the isopropyl methyl groups resonating at 21.34 and 21.38 ppm appeared in the form of a doublet of doublets (1:3:3:1) with $^1J_{C-H}$ = 126.2 Hz the lines of which were additionally split to form a multiplet with $^3J_{C^{14}-H^{13}}$ = 4.2 Hz and $^3J_{C^{13}-H^{14}}$ = 5.6 Hz. A doublet from C-11 is also due to ^1J = 126.2 Hz. The values of ^1J in the cases of C-4 and C-15 amounted to 157.4 and 176.9 Hz, respectively, while in the spectrum of the GP the C_{15}–H spin–spin coupling constant is 191.0 Hz [10]. Such a considerable change in this constant was due in the present case to the passage of the GP molecule from the dialdehyde into the dilactol state. The signals at 114.75, 114.83, 114.85, and 115.91 and at 140.65 and 140.67, assigned, respectively, to C-8 and C-1, remained singlets. Each of the four weak-field resonance lines at 156.27, 156.38, 156.39, and 156.51 ppm assigned to C-7 appeared in the form of doublets with $^3J_{C^7-H^{15}}$ = 2.8 Hz.

On the whole, the assignment of the signals of the carbon atoms of compound (II) was made on the basis of the results of experiments on selective $^{13}C-\{^1H\}$ double resonance, where $\{^1H\}$ were 4, 11, and 12-15, and also from the results of a comparative analysis of the values of their chemical shifts and those for gossypol [10] and hemigossypol [13].

In the ^{13}C NMR spectrum of the product of the transformation of GP in nondeuterated methanol, recorded in CH_3OH, four signals were observed, at 53.14, 54.60, 54.69, and 54.75 ppm, which are characteristic for the carbon atoms of methoxy groups. In the high-resolution spectrum the values $^1J_{C^{16}-H}$ = 143.6 Hz and $^3J_{C^{16}-H^{15}}$ = 5.6 Hz were observed. The observation of these signals, together with the characteristics of the ^1H NMR spectrum described above, also confirmed the presence of O-methyl functions in compound (II). Furthermore, it can be seen from Table 3 that carbon atoms C-15 and C-15' resonated between 109.27 and 109.62 ppm, i.e., approximately 10 ppm downfield as compared with what is customary for anomeric carbon atoms [18-21]. Such a considerable shift is explained satisfactorily if the size of the α-contribution of OCH_3 groups to the chemical shifts of the atom under consideration is taken into account.

Thus, in methanol, gossypol is converted from the dialdehyde form into the 15,15'-dimethyl ether of the stereoisomeric dilactol forms (II). If CD_3OD is used, the analogue with the 15,15'-di-OCD_3 functions is formed which interferes with the detection in the ^1H and ^{13}C NMR spectra of compound (II).

In a study of the properties of the transformation products of gossypol it was observed that after the evaporation of methanol and the dissolution of the residue in chloroform a compound was formed with a chromatographic mobility different form the initial one and with a change in the absorption maximum in the UV spectrum. Gossypol itself is fairly stable in chloroform. For a more detailed study of this process and the products formed in it we carried out the following experiment. A solution of a stabilized mixture of stereoisomeric dimethyl ethers of the dilactol forms of GP in CD_3OD previously frozen in liquid nitrogen was dried in a high vacuum. The dry residue was redissolved in CD_3OD. The ^1H NMR spectra of this and the initial solution proved to be identical, which showed the retention of the native state of the sample during the drying process.

The dry residue of the mixture of dimethyl ethers of the dilactol forms of GP prepared in this way was dissolved in $CDCl_3$, and after 15 min, the first ^1H NMR spectrum was recorded. The general pattern indicated a process of transformation that had already begun, since at this stage the spectrum contained a number of new signals in addition to resonance lines

corresponding to a mixture of dimethyl ethers of dilactol forms of GP. In subsequent spectra a rapid decrease in the intensity of the signals and an increase in that of the newly formed ones was observed. After 3 h from the moment of dissolution the spectrum already contained mainly the resonance lines corresponding to the new state of GP. After only a day, the process of change in the general pattern had practically ceased, and the most intense group of signals had the following characteristics: 1.53 ppm – doublet with ^3J = 7.2 Hz: methyls of isopropyl groups; 2.35 ppm – singlet: CH_3–11; 3.61 ppm – multiplet: H-12.

In the weak-field part of the spectrum there were three singlet signals of the same intensity at 7.40, 7.76, and 8.49 ppm. When small amounts of CD_3OD were added to the solution the singlet at 7.40 ppm disappeared. This demonstrated its propinquity to the hydroxy group. Two other singlets at 7.76 and 8.49 ppm, can be assigned unambiguously to the H-4 and H-15 protons, respectively, since any molecular transformation taking place with the participation of the lactol ring should be reflected most strongly on the H-15 chemical shift, as is actually observed in a comparison of Tables 1 and 2.

The results of an analysis of the spectral characteristics that have been described show that the main product of the transformation of the dilactol forms of GP in chloroform is dianhydrogossypol (DAG). In favor of this statement is also the ^1H NMR spectrum of an acetone solution of the DAG that we had obtained, in which the signals of the H-4 and H-15 protons were present at 7.92 and 8.82 ppm. These characteristics correspond well [2] to those of DAG obtained by Clark's method [14].

We also investigated the ^{13}C NMR spectra of a solution of the final products of the transformation of a mixture of the stereoisomeric dimethyl ethers of the dilactol forms of GP in $CDCl_3$. The assignment of the resonance lines according to the type of hybridization of the corresponding carbon atoms was made by a comparative analysis of the characteristics of the spectra using complete suppression of spin–spin coupling with protons and of high-resolution spectra.

The chemical shifts of the carbon atoms of the three methyl groups were close to one another, these carbon atoms resonating at 20.36 and 20.41 ppm. A doublet from C-12 appeared at 27.06 ppm. These four carbon atoms were characterized by the $^1J_{C-H}$ value of 127.6 Hz.

A detailed analysis of the weak-field part of the spectrum between 110.0 and 200.0 ppm showed that it was possible to see four sets of resonance lines in it. It is convenient to begin their consideration with a set consisting of the 11 best-separated intense signals the values of the chemical shifts of which for compound (IV) are given in Table 2. The relative amount of the component corresponding to these signals in the solution under investigation was ∿75–80%. In the high-resolution spectrum, signals at 123.72 and 150.26 ppm appeared in the form of doublets having components split additionally with $^1J_{C-H}$ = 158.1 and 208.8 Hz. According to the values of these two types of characteristics, the resonance lines under consideration could be assigned unambiguously to C-4 and C-15, respectively. A singlet located in the weakest field of the spectrum at 175.33 ppm could be assigned only to the C-7 carbonyl group of the aromatic nucleus involved in double conjugation, which also explained the value of the chemical shift of this signal [15-17].

The assignment of the other eight resonance lines of the naphthyl carbon atoms not bearing protons was made on the basis of a comparative analysis of the fine structure of the signals in the high-resolution spectra and those obtained under the conditions of selective suppression of spin-spin coupling with protons ($^nJ_{CH}$, where n > 1). Thus, on saturation with an additional radio frequency field of the H-15 transitions of DAG (8.49 ppm) in its ^{13}C NMR spectrum the fine-resolution structure of two signals changed. Doublets at 116.64 ppm ($^2J_{C^8-H^{15}}$ = 11.8 Hz) and 151.36 ppm ($^3J_{C^1-H^{15}}$ = 8.7 Hz) were converted into singlets, and a doublet of doublets at 118.97 ppm ($^3J_{C^9-H^4}$ = 8.0; $^3J_{C^9-H^{15}}$ = 4.9 Hz) into a doublet with $^3J_{C^9-H^4}$ = 8.0 Hz.

In another spectrum obtained with saturation of H-4 (7.76 ppm) the doublet of doublets at 118.97 ppm was converted into a doublet with $^3J_{C^2-H^{15}}$ = 4.9 Hz, the multiplet at 117.46 ppm ($^3J_{C^2-H^4}$ = 7.5; $^3J_{C^2-H^{11}}$ = 4.9 Hz) into a doublet of doublets with $^3J_{C^2-H^{11}}$ = 4.9 Hz, and the components of the doublet at 150.26 ppm (C-15) contracted with a sharp increase in their intensity.

On the basis of these changes with allowance for the observed values of the chemical shifts and $^3J_{CH}$ values it can be decided that the signals at 116.64, 151.36, 117.46, and 118.97 ppm belong to the carbons C-8, C-1, C-2, and C-9, respectively. It also follows from these experiments that the value of the long-range spin-spin coupling ^5J between C-15 and H-4 is 1.0 Hz, while ^2J between C-8 and H-15 is 11.8 Hz. The pattern of splitting of the signal at 136.3 ppm is characteristic for C-3 which experiences spin-spin coupling with the H-11 protons with ^2J = 5.6 Hz [10]. When a radio frequency field was applied to H-12 (3.61 ppm), doublets at 126.31 ($^3J_{C^{10}-H^{12}}$ = 5.6 Hz) and 149.78 ppm, ($^3JC^6-H^{12}$ = 5.6 Hz) were converted into singlets, and the broadened signal without indications of fine structure at 130.8 ppm contracted with the half-width decreasing from 17 to 14 Hz. These results, in the light of the values of the chemical shifts, permit the resonance lines at 126.31, 149.78, and 130.8 ppm to be assigned to carbon atoms C-10, C-6, and C-5, respectively.

The complete assignment of the resonance lines in the ^{13}C NMR spectrum of DAG made by the method described above, and also all the values of $^nJ_{CH}$ observed and found experimentally for compound (IV) are given in Table 3. The second set of signals in the ^{13}C NMR spectrum of the solution under investigation appeared between 115.0 and 152.0 ppm and also contained 11 weak singlets. Their chemical shifts are given in the "anhydro part" column in Table 3. They repeat, in the form of unilateral satellites the intense signals of the main set from DAG considered above; however, the differences in the chemical shifts between them are not the same and range from −0.08 to +1.20 ppm.

It must be mentioned that the fine-splitting structure of the weak signals in the high-resolution spectrum that are under consideration are completely identical with those of DAG (IV). It can be seen from Table 3 that on passing from (IV) to the given "anhydro part" the chemical shifts of the C-(1–4) and C-9 and C-10 nuclei formed by the methoxy-containing aromatic ring undergo paramagnetic shifts. The greatest difference (+1.20 ppm) is observed for C-3. A diamagnetic nature of the change is found for the C-(5–8) nuclei. On the basis of these facts it could be assumed that the appearance of this set of signals is possibly due to the formation of the atropoisomeric form (III) of DAG. However, in this case a complication arises in the interpretation of the causes of the appearance of another two sets of resonance lines each of which also includes 11 weak signals between 110.0 and 200.0 ppm in the spectrum under investigation. Here attention is directed to the pairwise closeness of the values of the chemical shifts of the corresponding carbon atoms. It is sufficient to dwell only on some of them.

Thus, for example, at 199.48 and 198.98 ppm there are singlets characteristic for the aldehyde carbonyl group of GP [10]. In the high-resolution spectrum they appear in the form of two doublets with the same $^1J_{C-H}$ value of 190.7 Hz. Another pair of singlets can be detected in a higher field at 111.61 and 111.91 ppm, and these are also split into doublets, with $^2J_{CH}$ = 18.0 Hz if the spectrum is recorded under conditions of the retention of spin-spin interaction with protons. Such a value of the constant is characteristic for C-8 of GP in the aldehyde form, which experiences spin-spin coupling with H-15. Practically all the pair singlets can be revealed similarly, even in the densest parts of the spectrum. The values of the chemical shifts for them are close to those for GP in the initial dialdehyde form (I), and they are given in the "aldehyde part" and "gossypol" columns of Table 3.

Thus, having considered the characteristics of three sets of weak resonance lines in the ^{13}C NMR spectrum under investigation we come to the conclusion that two of them (the "anhydro part" and the "aldehyde part") belong to monoanhydrogossypol and the third to gossypol. It is obvious that with this distribution of the signals the assumption of the possibility of the formation of an atropoisomeric DAG is deprived of a clear basis, since it cannot explain the presence of another two sets of resonance lines characteristic for the aldehyde form of gossypol. It is most likely that the dimethyl ethers of the dilactol stereoisomeric forms of GP (II) in chloroform are mainly converted into dianhydrogossypol, while monoanhydrogossypol (10-15%) and gossypol itself (5-10%) are formed as by-products. Apparently, this is newly formed gossypol, since its presence was not detected above in the ^{13}C NMR spectrum of product (II).

A solution of the final products of the transformation of the stereoisomeric lactol forms of the GP in chloroform, after preliminary freezing in liquid nitrogen, was dried in a high vacuum and the residue was dissolved in dry hexadeuteroacetone. The ^1H NMR spectrum of this solution contained the following resonance lines characteristic for DAG: 1.53 ppm – doublet with ^3J = 7.1 Hz: methyls of isopropyl groups; 2.35 ppm – singlet: CH_3-11; 3.68 ppm –

multiplet: H-12; 7.92, 8.15, and 8182 ppm – singlets: H-4, OH-6, and H-15, respectively.

In dry acetone, the spectrum under investigation and, therefore, DAG, were fairly stable. However, when small amounts of D_2O were added to the solution the spectrum began to change with a gradual appearance of new signals in different parts. After two days, the transformed spectrum corresponded practically completely to that of gossypol in acetone.

Thus, the cycle of transformations of gossypol is completed by the choice of solvents which can be represented by the following scheme:

$$\text{Gossypol (I)} \xrightarrow{\text{ROH}} \text{dilactol 15,15'-dimethyl ethers of GP (II)} \xrightarrow{\text{CHCl}_3} \text{dianhydrogossypol (IV)} \xrightarrow{(CH_3)_2CO+H_2O} \text{gossypol}.$$

It must be mentioned that in this cycle of molecular transformations the conversion of gossypol into derivative (II) (stage C) is preceded by the mixed aldehyde-lactol (15-OCH_3) form with one asymmetric center arising in the initial stage A, and then the mixture of aldehyde-lactol and dilactol (15,15'-di-OCH_3) compounds is converted into stage B.

A hypothesis was put forward previously [3] that the transformation of GP from the aldehyde form into the dilactol form is promoted by the appearance of single and doubly charged structures in an alkaline medium. Such a chemical condition is apparently not the only and exclusive one, as is shown by the formation of product (II) described in the present paper simply in methanol and also in other alcohols.

As applied to the observed cycle of mutual transformations of GP in solution, NMR spectroscopy on ^1H nuclei and on ^{13}C nuclei proved to be highly informative to equal degrees. The structural changes taking place are reflected in the spectral characteristics of practically all the protons of GP but in different degrees. In the spectra corresponding to the stage of conversion of GP into product (II) the changes in the chemical shifts of H-15, H-4, H-12, and H-11 are more characteristic, while those for the O–CH_3 groups unambiguously show their positions. Also important are the observed changes in the chemical shifts of these protons in the peculiarly changing ^1H NMR spectra, reflecting the conversion of product (II) with a dilactol nature into the dianhydro derivative (IV), as can clearly be seen from Table 1.

In the ^{13}C NMR spectra, attention is attracted by the increase in the spin-spin coupling constants $^1J_{C^{15}-H}$ in the series of compounds (II), (I), and (IV) in the same sequence: 176.9, 191.0, and 208.8 Hz, respectively. Consequently, the smallest value of this spectral characteristic corresponds to the dilactol form, the intermediate value to the dialdehyde structure, and the greatest value to dianhydrogossypol.

The C-15 carbon atom is extremely sensitive to the transformations of GP under consideration. The passage from the dialdehyde form to the 15,15'-dimethyl ethers of the dilactol forms of gossypol is accompanied by a diamagnetic change of the chemical shift of the carbon atom mentioned by ∿89 ppm, while for the purely dilactol structure one may expect even greater value of this difference – approximately 100 ppm.

In the transformation of the 15,15'-dimethyl ethers of GP dilactol into dianhydrogossypol, the chemical shifts of the majority of carbon atoms change very substantially. It is sufficient to point merely to C-15, C-7, C-1, C-4, and C-9, the resonance signals of which undergo paramagnetic shifts by 40.8, 18.9, 10.8, 8.9, and 8.8 ppm, respectively and which can be used as diagnostic spectral characteristics unambiguously showing the structural form of gossypol realized in a solution. Such striking changes in the spectrum are apparently due mainly to the presence of the carbonyl function conjugated with an α,β-unsaturated double bond, radically influencing the distribution of the labile physicochemical π-electronic system of the naphthyl nuclei of the dianhydrogossypol molecule.

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