PMR SPECTROSCOPIC INVESTIGATION OF VARIOUS SAMPLES OF GOSSYPOL

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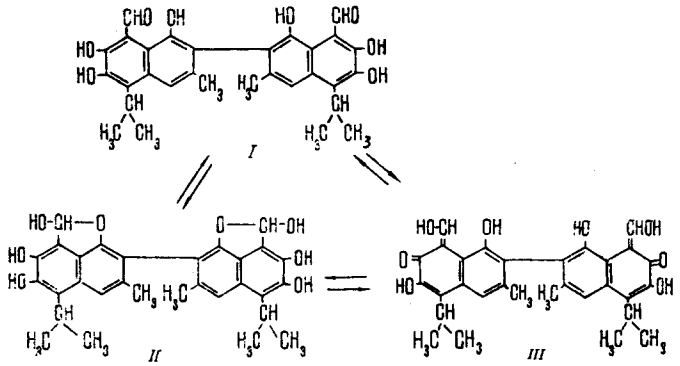
UDC 547.56

To explain the different chemical transformations of gossypol — a natural polyphenol — three tautomeric forms [1] are suggested, transformations between which are possible through prototropic rearrangements, leading to the aldehyde (I), lactol (II), and quinoid (III) forms:



The existence of each of the forms has been confirmed by the production of corresponding derivatives [1], but for gossypol itself it has not been possible to detect appreciable concentrations of the lactol and quinoid forms, although one of the reasons of the optical activity of (+)-gossypol found in some plants of the genus *Thespesia populnea* [2-4], which is close to the genus *Gossypium* (family Malvaceae), may be the presence of the asymmetric carbon of the lactol ring (II). This assumption has served as a basis for an attempt to detect the tautomeric forms of gossypol in various samples of it and to determine their quantitative ratio, all the more since in the PMR spectra, in addition to the main signals corresponding to the aldehyde form of gossypol, an additional signal has been detected at 7.38 ppm which has been ascribed to the proton of the lactol ring [4, 5]. The appearance of the additional signal is explained by the existence of gossypol in a form such that one half of the molecule is in the aldehyde form and the other in the lactol form. Other PMR spectroscopic investigations of (±)-gossypol [5, 6] and of (+)-gossypol [2-4] have shown its existence in a number of solvents only in the aldehyde form.

Assuming that the appearance of the additional signal may also be connected with a difference in the genera of plants used, we have made use of the method of isolating gossypol proposed by Datta et al. [5] in obtaining it from the bark of the roots of *Gossypium hirsutum* and from gossypol anthranilate.

For the investigation and the demonstration of the possible tautomeric forms, we used 13 samples of gossypol isolated from various sources and purified by various methods with the aid of various solvents (see Experimental Method). The results of the PMR spectroscopic study of these samples of gossypol are given in Table 1.

In some samples of gossypol, in actual fact, a signal is observed in the 7.20-7.25-ppm region that could be assigned to the proton of the lactol ring. Since the spectra of all the samples showed only one signal of an aromatic proton (H_4) at 7.66 ppm, which is common to the lactol and the aldehyde forms, the sum of the integral intensities of the lactol and

Division of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 249-252, March-April, 1976. Original article submitted July 14, 1975.

TABLE 1. Chemical Shifts of the Signals in the PMR Spectra of Various Samples of Gossypol

Sample No.	Solvent	δ, ppm			Ratio of the intensities of signals H_{addnl}/ H_{arom}
		H_{4arom}	H_{aldeh}	H_{addnl}	
1	Acetone	7,64	11,10	—	—
	Acetone + D_2O	7,70	11,10	—	—
2	Acetone	7,74	11,10	—	—
3	$CDCl_3$	7,64	11,04	—	—
	Acetone	7,54	11,01	—	—
4	$CDCl_3$	7,64	11,00	7,20	
	$CDCl_3$ + D_2O	7,66	11,04	7,20	<1
5	Acetone	7,70	11,04	7,25	
	Acetone + D_2O	7,70		7,25	>1
6	$CDCl_3$	7,68	11,02	7,24	
	Acetone	7,68	11,15	7,24	<1
7	Acetone	7,66	11,10	7,22	
8	$CDCl_3$	7,66	11,04	7,22	≪1
	Acetone	7,68	11,04	7,22	>1
9	Acetone	7,70	11,20	—	—
10	Acetone	7,70	11,20	7,24	>1
11	Acetone	7,72	11,20	—	—
12	Acetone	7,72	11,20	—	—
13	Diethyl ether	7,64	11,00	—	—

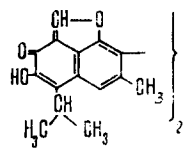
the aldehyde protons should be equal to the integral intensity of the H_4 signal, and the ratio of the integral intensities would determine the concentrations of the aldehyde and lactone forms. However, integration of the spectra showed that the integral intensity of the signal at 7.20-7.25 ppm is different in different samples, not infrequently exceeding the intensity of a whole proton unit (the H_4 signal), and the integral intensity of the signal of the aldehyde proton is equal to the intensity of the H_4 aromatic proton in all samples without exception.

Further, on analyzing the figures in the table and literature information [2-9], we found that the additional signals in the 7.20-7.25-ppm region appear in those cases where benzene or petroleum ether is used simultaneously with silica gel.

A comparison of samples 10 and 11, differing by their method of purification, confirms this hypothesis particularly clearly, all the more since the use of cellulose (sample 3) as adsorbent causes no such phenomenon. In addition, the participation of benzene in the appearance of the signal in the 7.20-7.25-ppm region is also shown by the fact that in all samples where the additional signal exists the addition of traces of benzene is accompanied by an increase in the intensity of just this signal. Furthermore, it must be mentioned that in an investigation of samples of gossypol obtained previously without the use of any adsorbents whatever and purified by recrystallization from various solvents, including benzene, no additional signals were observed in the 7.20-7.25-ppm region. All that has been said above does not permit us to consider the singlet in the 7.20-7.25-ppm region as the signal of the lactol proton.

Assuming that the additional signal can arise through the hemiacetal proton of anhydrogossypol [10], the probability of the formation of which on the reaction of a solution of gossypol with silica gel is quite possible, we recorded the PMR spectrum of this compound (IV).

However, in the PMR spectrum of anhydrogossypol the signal of the hemiacetal proton was observed at 8.74 ppm (the H_4 signal at 7.86 ppm).



IV

Hence, the analysis of the PMR spectra of various samples of gossypol has shown its existence in weakly polar solvents predominantly in the aldehyde form. The use of a polar solution, affecting the nature of the prototropic transitions, or the formation of derivatives may lead to a displacement of the equilibrium (I ⇄ II ⇄ III) and to the detection of

other tautomeric forms [11]. Thus, we have established that, in a solution of gossypol in dimethyl sulfoxide, lactol forms appear in dynamic equilibrium with the aldehyde form. The study of these tautomeric transformations is continuing at the present time.

EXPERIMENTAL METHOD

In the purification of samples of gossypol by means of column chromatography, we used as adsorbents silica gel of type L 40/100 (Chemapol, Czechoslovakia), cellulose (experimental sample obtained from NIIKhTTs), and polyamide [12].

The solvents were purified in accordance with the requirements set for solvents used in spectroscopy.

The PMR spectra were recorded in solutions of deuterated chloroform and acetone on a Varian XL-100 spectrometer, and the chemical shifts were determined in the δ scale relative to HMDS as internal standard.

Preparation of the Samples of Gossypol. Sample 1 was obtained from gossypol anthranilate and was recrystallized from a mixture of diethyl ether and petroleum ether (bp 40-60°C).

Sample 2 was obtained by passing sample 1 through a column of silica gel and eluting it with diethyl ether.

Sample 3 was obtained from gossypol anthranilate and purified by passage through a column of cellulose with elution by petroleum ether.

Sample 4 was obtained from gossypol anthranilate and was purified by passage through a column of polyamide with elution by benzene–chloroform–acetic acid (50:50:0.3).

Sample 5 was obtained from gossypol anthranilate and was purified by passage through a column of silica gel with elution by petroleum ether–diethyl ether (9:1).

Sample 6 was obtained from a benzene extract of the bark of the roots of the cotton plant (variety 108-F) and was purified by passage through a column of silica gel with elution by the petroleum ether–diethyl ether (9:1) system.

Sample 7 was obtained from a benzene extract of the bark of the roots of the cotton plant and was purified by three recrystallizations from a mixture of diethyl ether and petroleum ether.

Sample 8 was obtained by the passage of sample 3 through a column of silica gel with elution by the petroleum ether–diethyl ether (9:1) system.

Sample 9 was obtained by two recrystallizations of sample 8 from diethyl ether.

Sample 10 was obtained from an ethereal extract of the bark of the roots of the cotton plant and was purified by passage through a column of silica gel with elution by the petroleum ether–diethyl ether (9:1) system.

Sample 11 was obtained from an ethereal extract of the bark of the roots of the cotton plant and was purified by recrystallization from diethyl ether.

Sample 12 was obtained by passing sample 11 through a column of silica gel with elution by acetone.

Sample 13 was obtained from an ethereal extract of the bark of the roots of the cotton plant and was purified by passage through a column of silica gel with elution by diethyl ether.

All the samples of gossypol obtained were dried in vacuum (10 mm Hg) to constant weight.

SUMMARY

It has been shown on the basis of the results of a study of PMR spectra that, regardless of its source and method of purification, in weakly polar solvents gossypol exists predominantly in the aldehyde form.

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