Spectral Identification of the Ketol Tautomer of Gossypol

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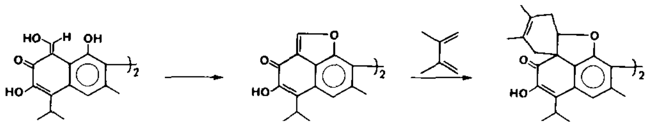
ABSTRACT

Nuclear magnetic reasonance studies have identified the ketol *lc* as the major tautomer of (±) gossypol in basic solvent systems. The hydroxymethylene proton of gossypol absorbs at δ 9.50 in aqueous sodium hydroxide. Acidification converts the ketol to the aldehyde tautomer *la*.

INTRODUCTION

In 1938, Adams and coworkers proposed that (±) gossypol derived from cottonseed was 8,8′-dicarboxaldehyde-1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-2,2′-binaphthalene (1). This structure was later confirmed by synthesis (2,3). To account for the diverse and unusual reactions of gossypol, they proposed that gossypol could exist in 3 tautomeric forms *la, lb,* and *lc* (4,5) (see Fig. 1). The aldehyde form *la* appeared to be the predominant form, based upon condensations with aniline and other ammonia derivatives in neutral solvents (6). IR (7) and NMR spectra (8) confirmed this supposition. A hexamethylether of gossypol was obtained by the action of dimethyl sulfate on gossypol in the presence of strong alkali (9). The hexamethylether of gossypol was stable to alkali and did not react with aldehyde reagents in neutral solvents; but condensations with aldehyde reagents (phenylhydrazine) did take place in the presence of acetic acid. Observations such as these suggested that 2 of the 6 methyl ether groups were of an acetal type, and the hemiacetal tautomer of gossypol *1b* was proposed to account for these results. The ketol tautomer *1c* was proposed, since gossypol readily loses 2 molecules of water on heating, producing anhydrogossypol. Anhydrogossypol reacts with dienes such as dimethylbutadiene (10) (Scheme I); gossypol also reacts with dienes with the simultaneous loss of two water molecules (1).



Scheme I.

Until recently, only the aldehyde tautomer *la* of gossypol had been observed spectroscopically. In 1972, Datta et al. (11) reported the isolation of (±) gossypol from *Thespesia populnea* (L.) Soland. The NMR sectrum of (±) gossypol from this source indicated a peak at δ 7.38. The chemical shift of this proton closely agreed with that of the hemiacetal proton (7.05) of the hexamethylether of (±) gossypol. The NMR spectrum of (±) gossypol also exhibited a peak at 11.10, caused by the aldehyde proton. Datta et al. interpreted this to mean that (±) gossypol existed as a mixture of tautomers of the aldehyde *la* and the hemiacetal *1b*. Integration of peak areas indicated that these existed in the ratio of 2:1, respectively, in chloroform.

We report here on the occurrence of the ketol tautomer of gossypol in aqueous alkaline solution, as determined by NMR spectroscopy. The ketol is unstable under neutral or acidic conditions and converts to the aldehyde tautomer.

MATERIALS AND METHODS

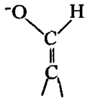
NMR spectra were measured on a JEOL MH-100 spectrometer at 23 C. Chemical shifts are in δ units (parts per million) downfield from tetramethylsilane (TMS) or 3-trimethylsilyl propionic acid sodium salt 2,2,3,3-d4 (TSP) (used in D_2O solutions), which were used as internal standards. Gossypol was obtained as the acetate, and acetic acid was removed, following the procedure of Adams (12). The NMR of the aldehyde tautomer of gossypol was recorded in deuterochloroform ($CDCl_3$). Other solvents, tetrahydrofuran (THF) and methanol (MeOH), were spectro quality. The ketol tautomer of gossypol was observed in a number of basic solvent systems. Two equivalents of base were sufficient to dissolve the gossypol, and the NMR spectrum remained unchanged even when a large excess of base was used. The NMR spectra of other model aldehydes (salicylaldehyde and 2,5-dihydroxybenzaldehyde) were recorded in both neutral and basic solvent systems.

RESULTS

The NMR chemical shift of gossypol in both $CDCl_3$ and $NaOD/D_2O$ is shown in Table I. The isopropyl septet was not observed in D_2O, because its absorption is in the same region as water (HOD). The methyl and aromatic protons of gossypol were shifted upfield only slightly in base. However, a drastic change was noted in the absorption of the aldehyde proton. The peak at 11.10, assigned to the aldehyde proton, disappeared (less than 1% remains), and a new peak appeared at 9.50 (s, 2H). When the solution was acidified to pH 8.0 with dilute HCl and extracted with $CDCl_3$, the peak at 9.50 was completely lost, and the normal aldehyde absorption at 11.10 was again observed. The same results were obtained when the basic gossypol solution was adjusted to pH 9 and extracted with a

TABLE I

NMR Spectra of Gossypol

Proton	$CDCl_3$	$NaOD/D_2O$
$\overset{CH_3}{\underset{CH_3}{>C}}$	1.54 (d)	1.48 (d)
CH_3	2.14 (s)	1.92 (s)
$H\text{-}CMe_2$	3.90 (sept)	
HO	5.93 (s)	
HO	6.40 (s)	
H-Ar	7.79 (s)	7.44 (s)
		9.50 (s)
CHO	11.10 (s)	
HO	15.08 (s)	

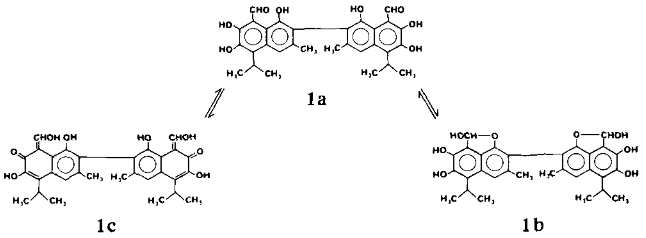


FIG. 1. Tautomers of gossypol.

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[1] ARS, USDA.

TABLE II

NMR Positions of Aldehyde Protons

Solvent	$CDCl_3$	THF	THF/NaOH[a]	CH_3OH-THF[b]	CH_3OH-THF/NaOH[a,b]	H_2O	H_2O/NaOH[a]
Salicylaldehyde	9.74	9.90	9.95	9.85	9.94		10.0
2,5-Dihydroxybenzaldehyde	9.79			9.80	9.86	9.67	9.88
Gossypol	11.11	11.14	9.46[c]	11.26	9.74[c]		9.50[c]

[a] The solution was treated with an aqueous sodium hydroxide solution.

[b] The solution was a mixture of methanol and tetrahydrofuran (9:1).

[c] Absorption caused by the proton of the hydroxymethylene group.

continuous liquid-liquid extractor (ether-water).

For comparative purposes, 2 model aldehydes (salicylaldehyde and 2,5-dihydroxybenzaldehyde) were studied. The absorption position of the aldehyde proton in a series of solvent systems was investigated (Table II). For example, in MeOH:THF (9:1), the aldehyde proton of salcylaldehyde absorbed at 9.90, and when aqueous base was added, the shift was downfield to 9.95. Similarly, a small downfield shift was observed for the aldehyde proton in 2,5-dihydroxybenzaldehyde in going from neutral to basic solution. A very small upfield shift (0.2 ppm) was noted with *o*-hydroxybenzylalcohol when the solvent was changed from $CDCl_3$ to $NaOD/D_2O$.

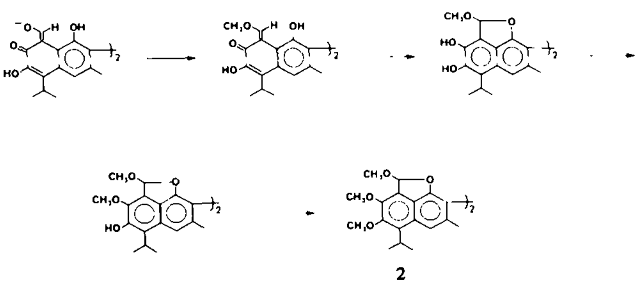
DISCUSSION

The NMR spectrum of gossypol in basic solvent systems agrees with the ketol structure of gossypol. An absorption in the 8.0-9.0 region is characteristic for the methylene proton of a number of ketols of the hydroxymethylene type. For example, the methylene proton of 2,hydroxymethylenecyclohexanone appears at 8.61 (13), and 2-hydroxymethylene 4-tertiaybutyl-6-benzylcyclohexanone is at 8.95 (14). The gossypol absorption at 9.50 ($NaOD/D_2O$) was thus caused by the methylene proton of the ketol. The slight downward shift to 9.50 for this proton was not unexpected, since it is coplanar and in close proximity to the aromatic ring. Thus, the NMR spectrum indicated that gossypol exists as an equilibrium mixture in basic solution, with the ketol tautomer being by far the predominant product. Acidification shifts the equilibrium back to the aldehyde form.

The large upfield shift between the NMR spectra of gossypol in neutral and basic solutions was not caused by a simple change in solvents. Salicylaldehyde and 2,5-dihydroxybenzaldehyde gave a small downfield shift in going from neutral to basic solutions.

The absorption at 9.50 is not caused by the lactol tautomer, because the lactol hydrogen absorbs at 7.38 ($CDCl_3$) (11). A large downfield shift for this proton is not expected when going from $CDCl_3$ to $NaOD/D_2O$. A study of *o*-hydroxybenzyl alcohol confirmed this. The chemical shift in the methylene group of *o*-hydroxybenzyl alcohol in going from $CDCl_3$ to $NaOD/D_2O$ was very small (0.2 ppm), and upfield rather than downfield, as would be required if the lactol proton *1b* were responsible for the absorption at 9.50.

Our findings explain the observations of Adams (15) and Datta (16). Adams reported that gossypol hexamethylether is predominantly in the diacetal form (*2*, Scheme II), while Datta reports significant amounts of both the dialdehyde hexamethylether *3* and the aldehyde-hemiacetal hexamethylether *4*. For the preparation of gossypol hexamethylether, Adams employed 20% potassium hydroxide in methanol as the base. Under these conditions, the ketol would be the predominant tautomer, providing the acetal hexamethylether (Scheme II) as the major product. This agrees with the sequence reported by Adams for the formation of gossypol hexamethylether: (a) gossypol dimethyl acetal, (b) gossypol dimethyl acetal-7,7′-dimethylether, and (c) gossypol dimethyl acetal-6,6′,7,7′-tetramethylether (1). That is, the hydroxyl proton of the hydroxymethylene group is the most acidic proton in the molecule and would be expected to methoxylate first. Ring closure to the acetal provides a reactive center at the C_7-hydroxyl, which would be expected to react next. Final methoxylation at C_6 provides the hexamethylether. Datta (16), employing less basic conditions (sodium carbonate in acetone), obtained a mixture containing significant amounts of dialdehyde hexamethylether *3* and aldehyde-hemiacetal hexamethylether *4*.



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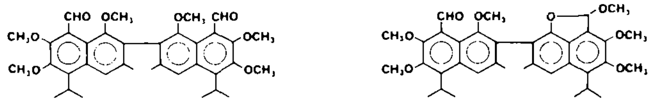

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Scheme II.

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