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Spectroscopic studies and PM5 semiempirical calculations of new hydrazone of gossypol with 3,6,9-trioxadecylhydrazine

Grzegorz Bejcar, Piotr Przybylski, Monika Walkowiak, Bogumil Brzezinski *

Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznan, Poland

Abstract

A new hydrazone of gossypol with 3,6,9-trioxadecylhydrazine (GHTO) has been synthesised and its structure has been studied by ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopy and PM5 semiempirical methods. The results have shown that the newly synthesised hydrazone exists in solution in the N-imine–N-imine tautomeric form, stabilized by several intramolecular hydrogen bonds among which the $O_7H \cdots N_{16}$ intramolecular hydrogen bond is the strongest. The structure of GHTO is visualized by the PM5 semiempirical calculations.
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1. Introduction

Gossypol 2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) is a well-known disesquiterpene showing great biological activity [1–10]. Very important groups of gossypol derivatives are its Schiff bases and hydrazones, which are characterized by significantly lower toxicity than gossypol [11,12]. It is also well known that gossypol can occur in three symmetrical tautomeric forms shown in Scheme 1. In contrast, Schiff bases and hydrazones of gossypol (Scheme 2) can be present in only two symmetrical tautomeric forms: imine–imine (N-imine–N-imine) and enamine–enamine (N-enamine–N-enamine) forms which are analogous to the aldehyde–aldehyde and ketol–ketol tautomeric forms of gossypol, respectively. Recently, we have studied the structures of several Schiff bases [13–22] and hydrazones [23–28] of gossypol. All these papers reported the occurrence of the enamine–enamine tautomeric forms of gossypol Schiff bases and N-imine–N-imine tautomeric forms of gossypol hydrazones both in solution and in the solid state. As a continuation of these studies, in the present paper we report the synthesis and

spectroscopic as well as semiempirical PM5 studies of a new hydrazone of gossypol with 3,6,9-trioxadecylhydrazine. The tautomeric structure of this new compound is discussed in detail.

2. Experimental

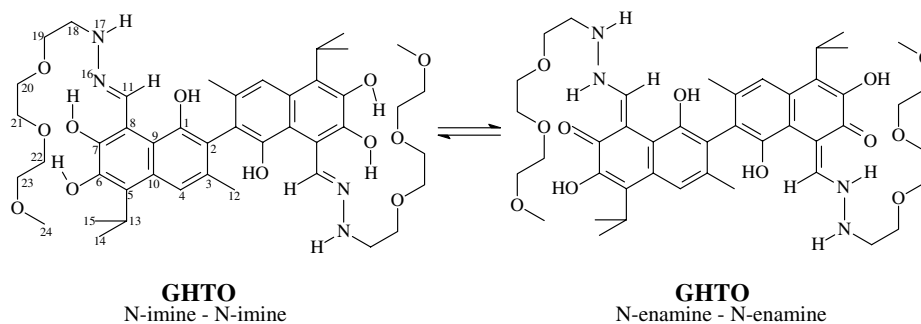### 2.1. Synthesis of 3,6,9-trioxadecylhydrazine

3,6,9-Trioxadecylchloride was synthesized from triethylene glycol monomethyl ether and PCl_3 in presence of pyridine (procedure from Ref. [29]). The purity of this compound was controlled by ^{1}H and ^{13}C NMR spectra.

3,6,9-Trioxadecylchloride [15.15 g (0.083 mol)], hydrazine monohydrate [41.5 g (0.83 mol)] and 15 cm^3 of ethyl alcohol were refluxed 6 h. After this time the solvent was evaporated under a reduced pressure from the reaction mixture. The residue was several times extracted by ethyl ether. The ether solutions collected were dried by freshly roast calcium oxide, the solvent was evaporated and the residue was distilled under reduced pressure. Pure 3,6,9-trioxadecylhydrazine is a colourless liquid with a characteristic amine-like odour. Boiling point 130–135 °C/3 mmHg. The purity of this compound was controlled by ^{1}H and ^{13}C NMR spectra.

* Corresponding author. Tel.: +48 61 829 1330; fax: +48 61 865 8008.
  E-mail address: bbrzez@main.amu.edu.pl (B. Brzezinski).
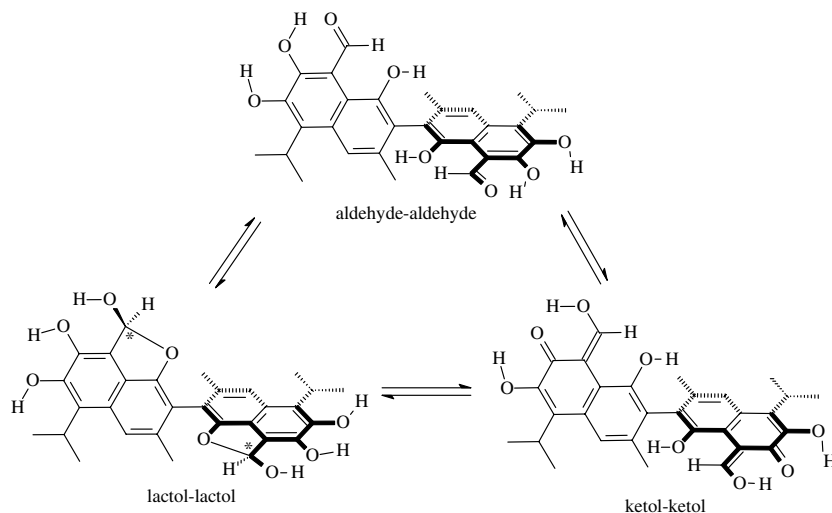
Scheme 1. Tautomeric forms of gossypol.

Scheme 2. Tautomeric forms of GHTO.

2.2. Synthesis of 3,6,9-trioxadecylhydrazone of gossypol (GHTO)

GHTO was synthesised by addition of a solution of 206.18 mg (1.15×10^{-3} mol) of 3,6,9-trioxadecylhydrazine in 5 cm^3 absolute ethanol to a solution of 300 mg of gossypol (5.78×10^{-4} mol) in 15 cm^3 absolute ethanol. The mixture was stirred under reflux for 5 h under argon atmosphere. After cooling of the mixture, the reaction product precipitated as a brown-yellow powder. The product was recrystallized from absolute ethanol and finally dried under reduced pressure. All manipulations were performed under argon atmosphere. Yield: 392 mg (80.7%). Melting point: 171–173 °C.

Elementary analysis $C_{44}H_{62}N_4O_{12}$: calculated, C = 62.99%, H = 7.45%, N = 6.68%; found: C = 62.95%, H = 7.42%, N = 6.71%.

2.3. FT-IR measurements

The FT-IR spectra of gossypol and GHTO were recorded in methylenechloride and chloroform (0.05 mol dm^{-3}), respectively at 300 K. Chloroform and methylenechloride

spectral-grade solvents were stored over 3 Å molecular sieves for several days.

A cell with Si windows and wedge-shaped layers was used to avoid interferences (mean layer thickness 170 μm). The spectra were taken with an IFS 66v/s FT-IR spectrophotometer (Bruker, Karlsruhe) equipped with a DTGS detector; resolution 2 cm^{-1}, NSS = 125. The Happ–Genzel apodization function was used.

All manipulations with the substances were performed in a carefully dried and oxygen-free glove box.

2.4. NMR measurements

The NMR spectra of GHTO were recorded in CDCl$_3$ using a Varian Gemini 300 MHz spectrometer. All spectra were locked to deuterium resonance of CD$_3$OD or CDCl$_3$, respectively. The error in ppm values was 0.01.

All ^{1}H NMR measurements 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 filling was used. Digital resolution = 0.2 Hz/point.

^{13}C NMR spectra were recorded at the operating frequency 75.454 MHz; pw = 60°; sw = 19,000 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. The ^{1}H and ^{13}C NMR signals have been assigned independently for each species using one- or two-dimensional (COSY, HETCOR) spectra. The ^{1}H NMR spectrum of GHTO was also measured after an addition of two drops of CD_3OD to identify the signals of OH and NH protons.

2.5. PM5 semiempirical calculations

PM5 semiempirical calculations were performed using the Win Mopac 2003 program [30–33]. For calculated hydrazone of gossypol full geometry optimization was carried out without any symmetry constraints.

2.6. Elementary analysis

The elementary analysis of GHTO was carried out on Vario ELIII (Elementar, Germany).

3. Results and discussion

The formula and the atom numbering of the hydrazone of gossypol with 3,6,9-trioxadecylhydrazine (GHTO) are shown in Scheme 2.

3.1. NMR studies

The chemical shifts of the signals observed in the ^{1}H and ^{13}C NMR spectra of GHTO are shown in Tables 1–3, respectively. These signals were assigned using one- and two-dimensional COSY and HETCOR spectra, respectively, as well as after the addition of CD_3OD to the probe.

In the ^{1}H NMR spectrum of GHTO (Table 1) the signals of the protons of OH and that of N—H groups are observed separately. After the addition of CD_3OD these signals vanish completely. The highest chemical shift of the $O_7H \cdots N_{16}$ intramolecular hydrogen-bonded proton is observed at 14.50 ppm. This chemical shift is up to now the highest when compared to that of the respective intramolecular hydrogen bond observed in the ^{1}H NMR spectrum of earlier studied gossypol hydrazones (14.43, 14.34 and 14.47 ppm) [23–25]. The signal of the O_6H proton is

found at 6.76 ppm, and its position is almost the same as in the spectra of all other hydrazones studied because this proton is engaged in the $O_6H \cdots O_7$ weak intramolecular hydrogen bond. The position of the O_1H proton signal at 5.78 ppm is slightly shifted toward higher frequencies relative to those of the respective signals observed in the spectra of other hydrazones. This shift is probably evoked by the formation of a weak intramolecular hydrogen bond between the O_1H proton and O_{23-24} oxygen atom from the oxaalkyl chain, which is in agreement with the ^{13}C NMR chemical shit of the C_1 atom as well as with the PM5 semiempirical calculations discussed below.

In the ^{13}C NMR spectrum of GHTO (Table 2) the signal of the C_7 atom is observed at 150.2 ppm. This chemical shift is comparable to that observed in the spectra of gossypol hydrazones studied previously (150.2 ppm [23–25]) and completely different from those of gossypol Schiff bases (172.3–173.0 ppm) [13–22]. Furthermore, the value of the chemical shift of about 150 ppm is characteristic of the C—OH carbon atoms of phenol molecules [34,35]. Thus, these results have clearly demonstrated that the proton in the $O_7H \cdots N_{16}$ intramolecular hydrogen bond is localized at the O_7 oxygen atom and for this reason the GHTO molecule exists in the solution as the *N*-imine–*N*-imine tautomeric form.

3.2. FT-IR studies

The FT-IR spectra of GHTO in chloroform and of gossypol in methylenechloride solutions are compared in Fig. 1a. The same spectra on the extended scales in the regions of the ν(OH) and ν(C=O, C=N) stretching vibrations are also shown (Fig. 13b and c, respectively). In all figures, the solid lines represent the spectra of GHTO and dashed-dotted lines the spectra of gossypol, for comparison.

In the spectrum of GHTO (Fig. 1b, solid line) the band assigned to the ν(OH) stretching vibrations is only slightly shifted to 3494 cm^{-1} relative to the band at 3502 cm^{-1} in the spectrum of gossypol. This indicates that the OH groups in the positions 1, 1′ in both compounds are involved in similarly strong hydrogen bonds. The broadened band assigned to the stretching vibrations of OH groups at the 6, 6′ positions and to the stretching vibrations of N_{17}—H and $N_{17'}$—H groups arises in the range

Table 1
^{1}H NMR chemical shifts (ppm) of GHTO in $CDCl_3$

Compound	Chemical shift (ppm)																
	O_1H	C_4H	O_6H	O_7H	$C_{11}H$	$C_{12}H$	$C_{13}H$	$C_{14}H\ C_{15}H$	$N_{17}H$	$C_{18}H$	$C_{19}H$	$C_{20}H$	$C_{21}H$	$C_{22}H$	$C_{23}H$	$C_{24}H$
GHTO	5.78 (bs)	7.68 (s)	6.76 (bs)	14.50 (bs)	9.58 (s)	2.10 (s)	3.86 (sept)	1.54 (d) 1.55 (d)	5.40 (s)	3.34 (t)	3.68 (t)	3.46[a] (t)	3.58[a] (t)	3.58 (t)	3.46 (t)	3.26 (s)
GHTO + CD_3OD	–	7.68 (s)	–	–	9.61 (s)	2.10 (s)	3.86 (sept)	1.53 (d) 1.56 (d)	–	3.34 (t)	3.68 (t)	3.46[a] (t)	3.58[a] (t)	3.58 (t)	3.46 (t)	3.27 (s)

s, singlet; bs, broad singlet; d, doublet; t, triplet; sept, septet.
[a] Assignment can be changed.

Table 2
^{13}C NMR chemical shifts (ppm) of carbon atoms of GHTO in CDCl$_3$ (gossypol moiety)

Compound	Chemical shifts (ppm)														
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}
GHTO	149.4	114.4	132.1	117.3	126.0	144.4	150.2	107.1	114.2	129.5	152.0	20.4	27.4	20.7	20.7

Table 3
^{13}C NMR chemical shifts (ppm) of carbon atoms of GHTO in CDCl$_3$ (oxalkyl moiety)

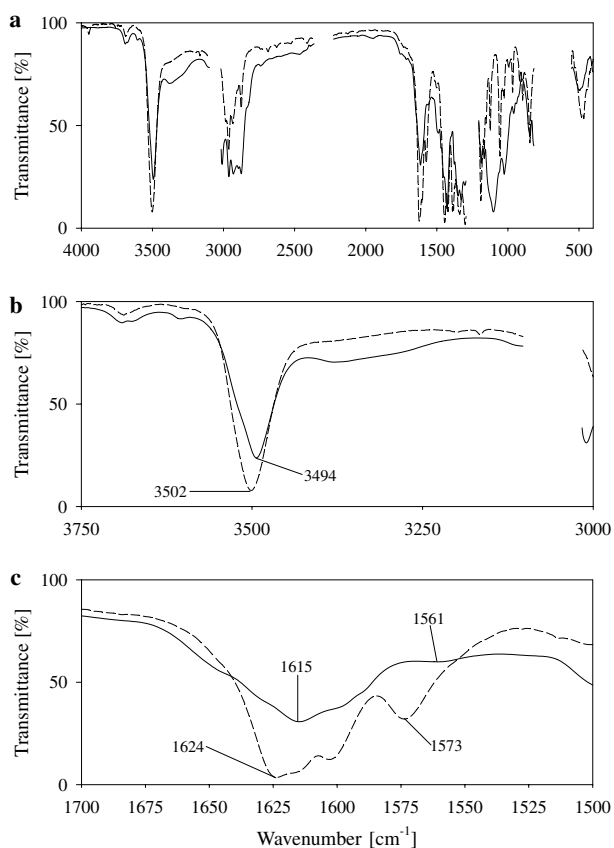| Compound	Chemical shifts (ppm)						
	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}
GHTO	49.6	68.3	70.4	70.5	70.4	71.8	58.9

Fig. 1. FT-IR spectrum of (——) GHTO in CHCl$_3$, and for comparison of (- - -) gossypol in CH$_2$Cl$_2$; (a) 4000–400 cm^{-1}, (b) 3750–3000 cm^{-1}, (c) 1700–1500 cm^{-1}.

3400–3200 cm^{-1}. The vibrations of the strongest O$_7$H···N$_{16}$ hydrogen-bonded proton are relatively well observed in the FT-IR spectrum in the region below 3000 cm^{-1}.

Details of the tautomeric structure of GHTO can be concluded from the region 1700–1500 cm^{-1}. In the spectrum of gossypol the v(C=O) vibration of the aldehyde group is observed at 1624 cm^{-1}. In the spectrum of GHTO this band vanishes completely and only one broad band, mainly caused by the overlaying of the v(C$_{11}$=N), v(C=C) and δ(N—H) vibrations, with a maximum at about 1615 cm^{-1} arises. This spectral feature indicates the forma-

tion of the N-imine–N-imine tautomeric form (analogous to the imine–imine form of gossypol Schiff base) within the GHTO molecule. The most important result confirming this conclusion is the presence of the band of v(C=C) vibrations characteristic of naphthalene ring, in the spectrum of gossypol at 1573 cm^{-1}. In the spectrum of GHTO molecule, this vibration is shifted to 1561 cm^{-1} due to the coupling of a lone electron pair on N$_{17}$ atom with the π electron system.

3.3. PM5 calculations

The calculated heats of formation for N-imine–N-imine and N-enamine–N-enamine tautomeric forms of GHTO molecule are −1445.89 kJ mol^{-1} and −1410.79 kJ mol^{-1}, respectively, indicating that the first tautomer is energetically favourable.

The hydrogen bond lengths and angles stabilising the N-imine–N-imine tautomeric form of GHTO are collected in Table 4 and its structure is shown in Scheme 3. The hydrogen bonds existing in this structure are marked by dots. The strongest hydrogen bond is the O$_7$···H—N$_{16}$ intramolecular one. The length of this bond is about 2.45 Å and its hydrogen bond angle is 153.6°, indicating that it belongs to the hydrogen bonds of medium strength. This fact is also in good agreement with the FT-IR and ^{1}H NMR spectroscopic results. Within the structure of GHTO two additional intramolecular hydrogen bonds are formed between the N$_{17}$—H and O$_1$—H proton donors and the oxygen atoms of the oxaalkyl chains. The parameters of these intramolecular hydrogen bonds indicate that they are of low strength. This finding is in very good agreement with the spectroscopic data discussed above. A comparison of the GHTO structure with that of the respective Schiff base of gossypol with 3,6,9-trioxadecylamine [15] demonstrates that the O$_7$···H—N$_{16}$ intramolecular hydrogen bond becomes stronger in the GHTO molecule and the oxaalkyl chains are hydrogen bonded in different ways due to the presence of the N$_{17}$—H proton donor group in the structure of hydrazone.

The dihedral angle in the calculated structure of (S)-GHTO, shown in Scheme 3 is +96.9 and this value is typical of all Schiff bases and hydrazones of gossypol studied.

Table 4
Interatomic distances (Å) and angles (°) of the hydrogen bonds within *N*-imine–*N*-imine tautomeric form of GHTO.

Compound	Hydrogen bond length (Å) [angles (°)]				
	O_7—H$\cdots$N$_{16}$	O_1—H$\cdots$O$_{23-24}$	N_{17}—H$\cdots$O$_{19-20}$	O_6—H$\cdots$O$_7$	C_{11}—H$\cdots$O$_1$
GHTO	2.45 [153.6]	2.68 [141.4]	2.86 [103.2]	2.82 [115.7]	2.59 [129.8]
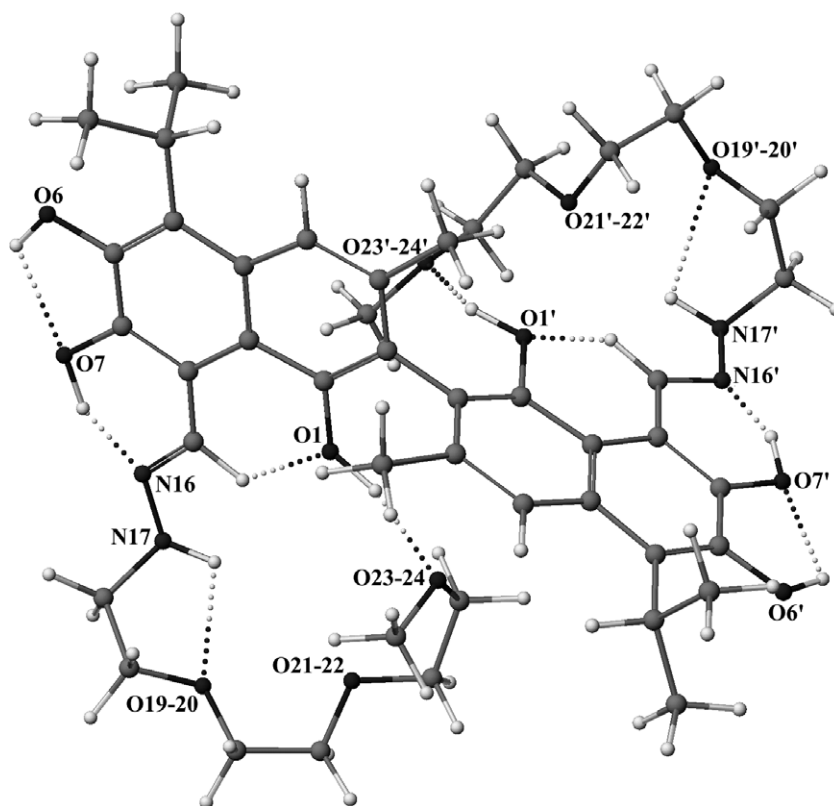
Scheme 3. The most probable structure of (*S*)-GHTO in the *N*-imine–*N*-imine tautomeric form calculated by PM5 method (WinMopac 2003).

Acknowledgements

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