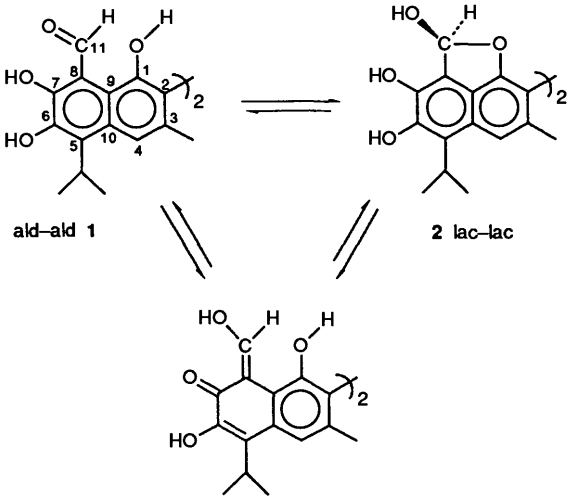
Spectroscopic and Kinetic Studies of the Aldehyde–Lactol Tautomerization of Gossypol in Solution

Bronislaw Marciniak,* Grzegorz Schroeder, Halina Kozubek and Bogumil Brzezinski
Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Poland

^{1}H NMR spectra of gossypol in C_2H_5OD solution show that an equilibrium between aldehyde **1** and lactol **2** tautomers is formed and that the aldehyde tautomer is predominant, whereas in C_2H_5OD–DCl solution (pH $\leqslant$ 1) only the lactol form is observed. UV–VIS absorption spectroscopy studies demonstrate that in ethanol and ethanol–HCl solutions gossypol shows different spectra which can be attributed to both tautomers **1** and **2**. The influence of various solvents on the $\mathbf{1} \rightleftharpoons \mathbf{2}$ equilibrium is studied by means of UV–VIS absorption spectroscopy and the results are compared with those of ^{1}H NMR studies. Furthermore, the decrease of the absorbance at the long wavelength band in the spectrum of **1** is used to determine the kinetic parameters of aldehyde–lactol tautomerization in ethanol–HCl solution (pH $\leqslant$ 3): $k_{298} = (2.14 \pm 0.03)10^{-2}$ dm^3 mol^{-1} s^{-1}; $\Delta H^{\neq} = 64.0 \pm 3.4$ kJ mol^{-1}; $\Delta S^{\neq} = -63 \pm 11$ J mol^{-1} K^{-1}; and the value of kinetic isotope effects, $k_H/k_D = 5.6$ at 55 °C. A mechanism for aldehyde–lactol tautomerization of gossypol is proposed.

Gossypol, 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-2,2′-binaphthalene-8,8′-dicarbaldehyde, a yellowish compound present in various parts of cotton plants, is of great interest because of its biological importance, *e.g.* contraceptive and toxic activities.[1–3] For these reasons extensive studies of chemical and physical properties of gossypol in solution have recently been carried out.[4–11] NMR and IR spectroscopy studies proved that gossypol can occur in three tautomeric forms in solution (Scheme 1). The aldehyde tautomer, **1**, was observed in chloroform and dichloromethane solutions, whereas the lactol was found to be the main form of gossypol in dimethyl sulphoxide (DMSO).[8,9] In other solvents an equilibrium between aldehyde and lactol tautomers was observed depending on the nucleophilicity of the solvent.[8] In NaOH–H_2O solution or as the tetrabutylammonium salt of gossypol, the ketol is the main tautomeric form.[8,9,12,13]



ald–ald **1**

2 lac–lac

3 ket–ket

Scheme 1

The stability of gossypol dissolved in various solvents and at different pH has been studied recently.[4,11,14] It was shown that the UV–VIS absorption spectra of gossypol are changed drastically in acidic ethanol solutions (pH < 4).[11] In this paper the spectroscopic and kinetic evidence for the aldehyde–lactol tautomerization of gossypol in solution will be presented and discussed.

Experimental

Yellow microcrystalline pure gossypol obtained from the Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Tashkent, USSR, was recrystallized twice from hexane (m.p. 180–181 °C) (Found: C, 69.3; H, 5.7. Calc. for $C_{30}H_{30}O_8$: C, 69.5; H, 5.79%). All solvents and reagents were spectroscopic grade and used without further purification. The appropriate solutions of NaCl and HCl in ethanol were used as pH buffers.[15] For each solution, the pH was measured according to the procedure described in ref. 15.

UV–VIS absorption spectra were recorded using a Specord M-40 UV–VIS (Zeiss) spectrophotometer. ^{1}H NMR spectra were measured by means of a Tesla MHZ-80 spectrometer and were calibrated against Me_4Si as an internal standard.

The kinetic runs were carried out using a Specord UV–VIS (Zeiss) spectrometer with the cell block thermostat maintaining the temperature to within ±0.1 °C. The kinetic runs were completed under pseudo-first-order conditions with the acid concentration in *ca.* 40- to 2000-fold excess. Pseudo-first-order rate constants (k_{obs}) were calculated from the traces of absorbance at the long wavelength absorption band of gossypol (at 373 nm) *vs.* time using the Guggenheim method and the second-order rate constants (k) were calculated by a linear least-squares fit of the variation of k_{obs} with H^+ concentration. The activation parameters were calculated by a linear least-squares fit of ln (k/T) *vs.* T^{-1} according to the Eyring equation.[16]

Results and Discussion

The Aldehyde–Lactol Tautomerization of Gossypol in Solution; ^{1}H NMR and UV–VIS Absorption Spectroscopy Studies.—We have recently found that an equilibrium between aldehyde and lactol tautomers of gossypol is formed depending on the nucleophilicity of the solvent.[8] In CD_3OD solutions both aldehyde and lactol tautomers have been observed. The same is true in the case of the C_2H_5OD solution of gossypol because all characteristic proton signals of the aldehyde and lactol tautomers are observed in the ^{1}H NMR spectrum.

Results taken from the ^{1}H NMR spectra of gossypol in C_2H_5OD and C_2H_5OD/DCl solutions are summarized in Table 1. When the small amount of DCl–C_2H_5OD mixtures was added to the C_2H_5OD solution of gossypol the singlets for H–C(11) (aldehyde group) and H–C(4) protons, which are seen in C_2H_5OD solution, are no longer observed. Instead of these two signals two new signals for the H–C(4) proton and two new

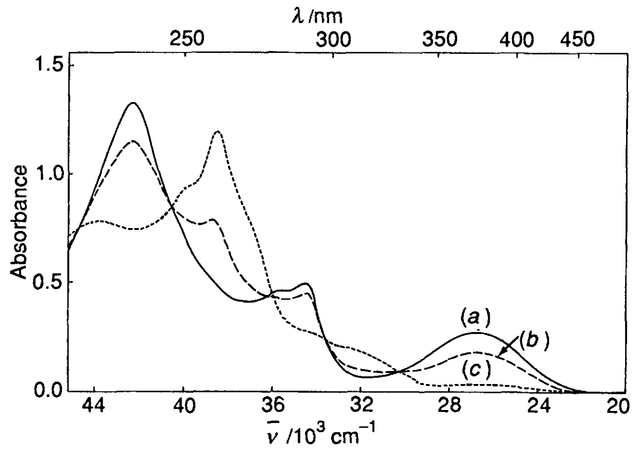


Fig. 1 Absorption spectra of gossypol (c = 1.5 × 10^{-5} mol dm^{-3}, l = 1 cm) in ethanol (a) and in ethanol–HCl solution taken after 20 min (b) and 60 min (c) in the dark at room temperature

Table 1 Chemical shift (ppm) of the proton signals of aldehyde and lactol tautomers of gossypol

	Solvent			
	C_2H_5OD		C_2H_5OD–DCl	
Proton	Aldehyde	Lactol	Aldehyde	Lactol
$(CH_3)_2C$	1.55(d)		—	1.51
CH_3	2.09	2.11	—	2.24, 2.19
H–CMe_2	3.92(sept.)		—	masked
H–C(11)	11.32	7.08	—	6.88, 6.87
H–C(4)	7.72	7.29	—	6.96, 6.93

Table 2 Long wavelength absorption band of gossypol ($c \approx 10^{-5}$ mol dm^{-3}) and amounts of aldehyde tautomers in the $\mathbf{1} \rightleftharpoons \mathbf{2}$ equilibrium in various solvents

Solvent	λ_{max}/ nm	ε'^{a}/ dm^3 mol^{-1} cm^{-1}	Aldehyde[b] (%)	B^c	Aldehyde[d] (%)
$CHCl_3$	364	20 100	100	14	100
CH_3CN	367	17 700	88	160	67
CH_3OH	375	17 200	85	218	53
DMSO	379	4 100	20	362	17

[a] Molar absorption coefficient calculated using the absorbance of the long wavelength absorption band and initial gossypol concentration dissolved in a particular solvent. [b] Amount of aldehyde tautomer estimated from the absorbance of the long wavelength band (initial concentration of gossypol *ca.* 10^{-5} mol dm^{-3}). [c] B is the nucleophilicity of the solvent.[8] [d] Amount of aldehyde tautomer calculated from ^1H NMR results[8] (initial concentration of gossypol 5 × 10^{-2} mol dm^{-3}).

signals for the H–C(11) proton are found (Table 1) indicating two (±) diastereoisomers of the lactol tautomers in the solution. Both the restricted rotation around the C(2)–C(2) bond and the chirality of the C(11) atom may be the reason for the presence of diastereoisomers of the lactol tautomer. This problem has already been discussed for the ^1H NMR spectrum of the lactol form of gossypol in DMSO solution.[8] All these spectral features prove that a gossypol molecule can occur only as the lactol tautomer in the C_2H_5OD–DCl solution (pH ≤ 1).

The UV–VIS absorption spectrum of gossypol consists of three well separated bands, and they are well reproduced by semiempirical INDO/S CI calculations.[11] The position of the absorbance maxima and the values of the molar absorption coefficient significantly depend on the solvent used. The UV absorption spectra of gossypol in ethanol and ethanol–HCl solutions are presented in Fig. 1. Addition of acid to an ethanol solution of gossypol leads to a time-dependent decrease in the intensity of the three well separated bands of gossypol at 373, 290 and 236 nm, and formation of new bands at *ca.* 310 and 236 nm with four isosbestic points (Fig. 1). On the basis of the results of ^1H NMR studies (*vide supra*) the spectrum of Fig 1(c) can be attributed to the lactol tautomer, and the spectrum of Fig 1(a) mainly to the aldehyde form.

The solvent effects on the long wavelength absorption band of gossypol are summarized in Table 2. Since this band can be attributed mainly to the absorption of the aldehyde tautomer, the total amount of **1** in the equilibrium $\mathbf{1} \rightleftharpoons \mathbf{2}$ can be estimated from the value of absorbance at λ = 364 nm [assuming 100% of **1** in chloroform, as it was proved in the ^1H NMR studies[8] (Table 2)]. It is important to note that the changes in the molar absorption coefficient at the long wavelength absorption band for the aldehyde tautomer with solvent have been neglected in the estimation of the amount of **1** in various solvents. The results obtained by means of UV spectroscopy do not give a quantitative correlation of the percentage of aldehyde tautomer with nucleophilicity, B, of the solvent, as in the case of ^1H NMR studies.[8] However, they show a decrease of the total amount of the aldehyde form with increasing nucleophilicity of the solvent. The differences between the results obtained by these two techniques can also be explained in terms of the experimental conditions used in the experiments; the UV absorption measurements have been performed for the initial gossypol concentration of 10^{-5} mol dm^{-3}, whereas the ^1H NMR measurements were for a concentration of *ca.* 500 times higher.

Since the influence of the various types of solvent on the intensity of the electronic transitions of tautomers **1** and **2** of gossypol is not known, the results presented in Table 2 do not lead to quantitative correlation of the percentage of the aldehyde form with the nucleophilicity of the solvent. However, the decrease of the long wavelength absorption at 373 nm can be used to determine the kinetic parameters of aldehyde–lactol tautomerization in the acidic ethanol solutions (pH ≤ 3).

Kinetics of Aldehyde–Lactol Tautomerization of Gossypol in the Alcohol–HCl Solutions.—^1H NMR and UV spectra have shown that the aldehyde–lactol tautomerization of gossypol ($\mathbf{1} \rightarrow \mathbf{2}$) occurs in the C_2H_5OH–HCl solution (*vide supra*). Since the compound studied is of the binaphthyl type one would expect the formation of asymmetric aldehyde–lactol forms of gossypol. These forms have not been observed spectroscopically (see also refs. 8–10). Therefore, the tautomerization of gossypol was treated as if it were a **1** (ald–ald)→**2** (lac–lac) reaction without participation of the asymmetric (ald–lac) form. In addition, the results of INDO/S CI calculations[11,17] have shown that there is no significant electronic coupling between two naphthyl units in the gossypol molecule and in its tautomeric forms.

The progress of the reaction was studied by determination of the aldehyde tautomer concentration monitored by the decrease of absorbance at the long wavelength band of gossypol at 373 nm. It was found that $\mathbf{1} \rightarrow \mathbf{2}$ tautomerization is the first-order reaction for both substrates, *e.g.* aldehyde and acid. The applied excess of H^+ concentration allowed determination of the pseudo-first-order rate constant, k_{obs}. Values of k_{obs} were measured for various concentrations of H^+ in solution and at several temperatures and were fitted to eqn. (1) to calculate the second-order rate constant (k) of aldehyde–lactol tautomerization.

$$k_{obs} = k[H^+] \qquad (1)$$

The results are shown in Table 3. The activation parameters derived from the second-order rate constant, k, are summarized in Table 4.

Table 3 Rate constants for the aldehyde–lactol reaction of gossypol in buffered acidic ethanol solution (initial concentration of gossypol 3×10^{-5} mol dm^{-3})

T/°C	pH	$k^{a}_{obs}/10^{-3}$ s^{-1}	$k/10^{-2}$ dm^3 mol^{-1} s^{-1}
(i) For HCl:			
15	0.88	1.0	—
25	0.88–3.0	2.8–0.038	2.14 ± 0.03
35	0.88–3.0	5.6–0.072	4.26 ± 0.05
45	0.88–2.5	14.4–0.25	11.1 ± 0.2
55	0.88–2.5	31.7–0.96	23.8 ± 0.2
(ii) For DCl:			
55	0.88–3.0	5.8–0.13	4.27–0.08

[a] Correlation coefficient, $r \geqslant 0.994$.

Table 4 Summary of reaction parameters for the aldehyde–lactol tautomerization of gossypol in the C_2H_5OH–HCl solutions

Parameter	Value	Unit
k_{298}	$(2.14 \pm 0.03) \times 10^{-2}$	dm^3 mol^{-1} s^{-1}
$\Delta H^{\ddagger}$	64.0 ± 3.4	kJ mol^{-1}
$\Delta S^{\ddagger}$	-63 ± 11	J mol^{-1} K^{-1}
$\Delta G^{\ddagger}_{298}$	82.8 ± 3.4	kJ mol^{-1}
k_H/k_D	5.6 (at 55 °C)	—

Aldehydes are known to react with alcohols to form acetals in acid solutions.[18] However, as shown by UV–VIS absorption and NMR studies, there is no reaction of gossypol with ethanol in the presence of HCl under the experimental conditions used (no acetal formation). Additionally, the aldehyde–lactol tautomerization was also observed in various acidified polar solvents, *e.g.* DMSO and DMF. Thus, in the reaction studied, alcohol must be treated as a solvent but not as a reaction substrate.

Since **1**→**2** tautomerization of gossypol in C_2H_5OH–HCl solution cannot be a one-step process, one should first determine which process, *i.e.* the protonation of **1** or the ring-closing process, describes the rate of **1**→**2** tautomerization. Therefore, an extra series of kinetic runs was carried out using deuteriated acid (DCl) to study the isotope effects. These results are also presented in Table 3 and values of the kinetic isotope effects in Table 4.

It was found by McClelland *et al.*[19] that the formation of cyclic hemiacetals in acidified water solutions occurred by way of two competing mechanisms. For the acid-catalysed **1**→**2** tautomerization of gossypol (the lactol form of gossypol can be treated as a cyclic hemiacetal) these two mechanisms may be written as shown in reactions (2) and (3). Reaction (2), involving the protonated lactol as an intermediate, presents the mechanism in which the simultaneous protonation and cyclization process is the rate-determining step followed by the equilibrium between the protonated lactol and lactol form of gossypol. According to the second mechanism [reaction (3)], the diffusion-controlled equilibrium process of aldehyde protonation[20] is followed by the formation of lactol with simultaneous deprotonation.

For the **1**→**2** tautomerization of gossypol a third possible mechanism involving both protonated aldehyde and lactol as intermediates can also be considered [reaction (4)]. In this case, the cyclization process is predicted to be the rate-determining step.

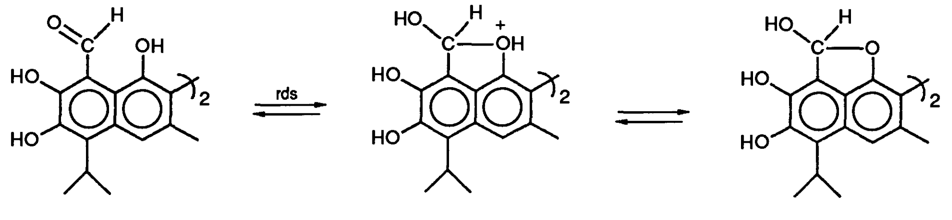
The aldehyde oxygen in the tautomer **1** is expected to be a site for protonation. This is confirmed by the distribution of electron densities in **1** obtained by the semiempirical INDO/S CI calculations.[17]

The obtained values of the activation parameters and the kinetic isotope effect (Table 4) suggested that the **1**→**2** tautomerization occurs *via* the first mechanism [reaction (2)], in which the simultaneous protonation and cyclization process is the rate-determining step. The other mechanisms [reactions (3) and (4)] may be excluded, since they cannot explain the large value of the kinetic isotope effect, k_H/k_D, and the negative values of the entropy of activation in ethanol solution [for reaction (3)]. Since, in our spectroscopic measurements, no intermediates were observed (see Fig. 1), the kinetic isotope effect and thermodynamic data were the only arguments in support of the mechanism of aldehyde–lactol tautomerization of gossypol.

The free enthalpy of activation $\Delta G^{\ddagger}_{298} \approx 80$ kJ mol^{-1} is typical of the reaction of proton transfer in protic solvents.[21] The negative value of the entropy of activation can be explained by a strong interaction of the transition state with the solvent; *i.e.* the stabilization of the transition state by the solvent is stronger than that of the initial state.

The important role of the solvent in the formation of the transition state for the simultaneous protonation and cyclization reaction [rate-determining step, reaction (2)] is verified by the determined value of the solvent isotope effect, $k_{C_2H_5OD}/k_{C_2H_5OH} = 1.3$. This value is consistent with those predicted for the acid-catalysed reaction in protic solvents (1.1–2.3).[22]

The influence of solvent on the rate constant of **1**→**2** tautomerization was also studied for various types of alcohol. It was found that the pseudo-first-order rate constants, k_{obs}, depend significantly on the types of alcohol used: methanol (100) > propan-2-ol (29) > ethanol (2) ≫ *tert*-butyl alcohol (0). The relative values of k_{obs} shown in parentheses were determined at 27 °C for a constant amount of HCl added to



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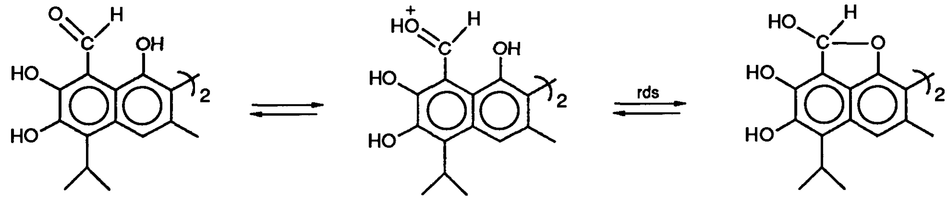

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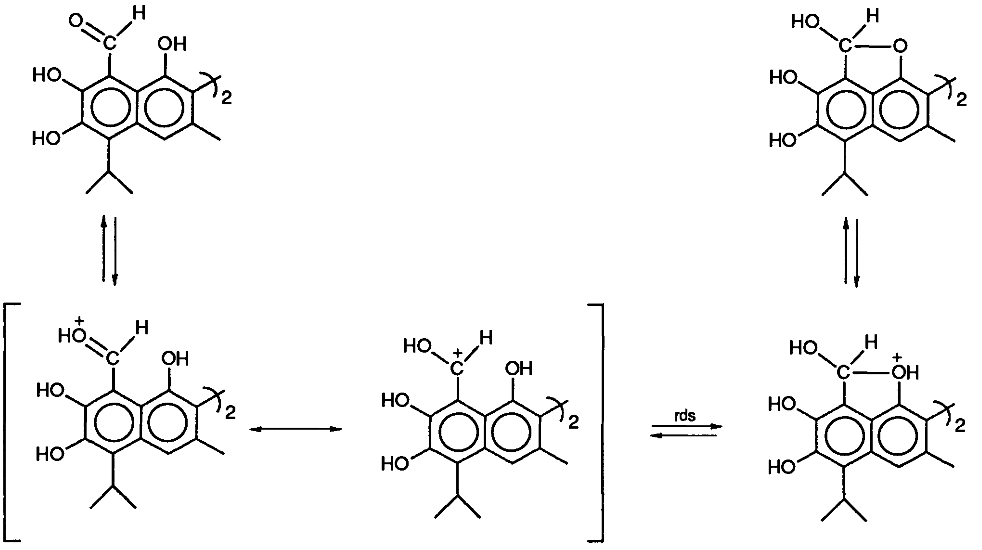

(4)

the gossypol solutions (k_{obs} in methanol was taken as 100). It was found that in *tert*-butyl alcohol (relative permittivity 12.5) **1**→**2** tautomerization did not take place. The reaction occurred in alcohols with higher values of relative permittivity. A strong dependence of the reaction rate on the polarity of the alcohol, as well as on the type and order of the alcohol, may result from a competition between protonation of solvent and substrate.

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References

1 R. Adams, T. A. Geissman and J. D. Edwards, *Chem. Rev.*, 1960, **60**, 555.
2 W. N. Leung and W. W. Tso, *Abstr. Chin. Med.*, 1988, **2**, 233.
3 M. B. Abou-Donia, *Residue Rev.*, 1976, **61**, 125.
4 A. A. Nomeir and M. B. Abou-Donia, *J. Am. Oil. Chem. Soc.*, 1982, **59**, 546; 1985, **62**, 87.
5 R. D. Braun, *Electrochim. Acta*, 1987, **32**, 459.
6 A. A. Tyshenko, S. D. Gusakova, I. P. Nazarova and A. I. Gluslenkova, *Khim. Prir. Soed.*, 1987, 354.
7 W. N. Leung, C. L. Au and W.-W. Tso, *Adv. Contracept. Deliv. Syst.*, 1985, 47.
8 B. Brzeziñski, J. Olejnik, S. Paszyc and T. F. Aripov, *J. Mol. Struct.*, 1990, **220**, 261.
9 B. Brzeziñski, J. Olejnik and S. Paszyc, *J. Mol. Struct.*, 1990, **239**, 23.
10 B. Brzeziñski, S. Paszyc and G. Zundel, *Chem. Phys. Lett.*, 1990, **167**, 7.
11 B. Marciniak, H. Kozubek, J. Koput and S. Paszyc, *Z. Naturforsch.*, 1990, **45**, 179.
12 F. G. Kamaer, N. I. Baram, A. I. Ismailov, V. B. Leontyev and A. Sadykov, *Izw. Acad. Nauk SSSR, Ser. Khim.*, 1979, 1003.
13 R. D. Stipanovic, A. A. Bell and C. R. Howell, *J. Am. Oil Chem. Soc.*, 1973, **60**, 462.
14 D.-H. Jiang, H.-B. Shen, X.-B. Yuang and L.-H. Qian, *Acta Pharm. Sin.*, 1986, **21**, 551.
15 D. D. Perin and B. Dempsey, *Buffers for pH and Metal in Control*, Chapman and Hall, London, 1974, ch. 6.
16 K. Schwetlick, *Kinetische zur Untersuchung von Reactions-Mechanizmen*, VEB *Deutscher Verlag der Wissenschaften*, Berlin, 1971.
17 B. Marciniak, J. Koput, 1990, unpublished results.
18 K. P. C. Vollhardt, *Organic Chemistry*, Freeman, New York, 1987, p. 636.
19 J. Harron, R. A. McClelland, C. Thankachan and T. T. Tidwell, *J. Org. Chem.*, 1981, **46**, 903.
20 R. B. Martin, *J. Am. Chem. Soc.*, 1962, **84**, 4130.
21 R. P. Bell, *The Proton in Chemistry*, Chapman and Hall, London, 1973.
22 J. P. Bellobono, P. Beltrame, M. G. Cattania and M. Simonetta, *Tetrahedron Lett.*, 1968, **1**, 2673.

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