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MECHANISM OF H/D EXCHANGE IN 5-(p-NITROBENZYL)BARBITURIC ACID

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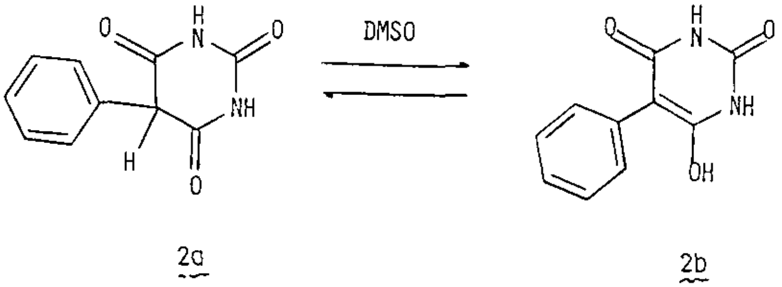
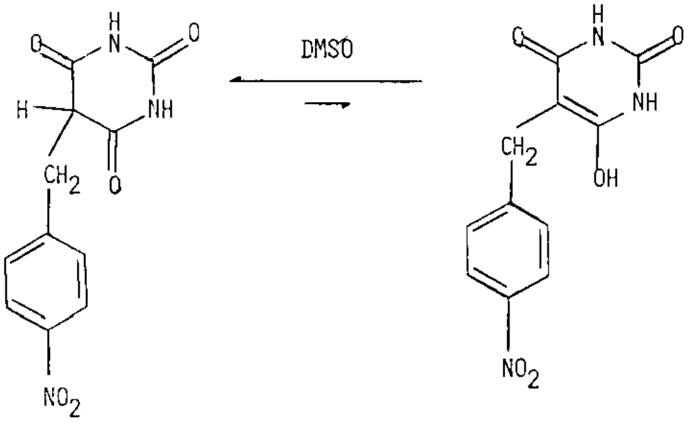
Abstract - Title compound 1 and related systems exist in DMSO solution as a tautomeric mixture of enol and keto forms which were easily identified by ^{13}C nmr spectroscopy. Base-catalyzed H/D exchange of 1 occurs at both the C-5 and the methylene group. A mechanism to account for the novel H/D exchange at methylene position is proposed.

Understanding of physical properties of barbiturates is essential for proper interpretation of their biological activity.[1] Structure, dissociation constants and tautomerism are some of the factors which probably contribute significantly to their metabolic efficiency at the receptor sites and define their mode of action.[2,3]

Recently, we reported that 5-phenylbarbituric acid (2) exists in the enol form, 2b, in the solid state and as a mixture of enol and keto, 2a, forms in DMSO solution.[4] It was the first barbiturate reported to exist completely in the enol form in the solid state and suggested that tautomeric equilibria may play an important role in the biochemistry of barbituric acid derivatives.



During the course of our investigation of the influence of substituents on the tautomeric equilibria of benzylbarbituric acids, we prepared 5-(p-nitro- benzyl)barbituric acid (1). Attempted purification of 1 with saturated sodium bicarbonate solution produced an orange solid, 3. The ^{13}C nmr spectrum showed eight absorptions with chemical shifts which closely resembled those of the enol form of the 5-phenyl derivative (2b) (See Table 1). These results indicated that we may have isolated the pure enol tautomer of 1 in a form of a sodium salt of its enolate anion. The proton-coupled ^{13}C spectrum of starting material 1 showed several broadened carbon resonances in the aromatic region as well as the two sets of signals of unequal intensity at 49.1; 32.0 ppm and at 88.0; 27.3 ppm indicating clearly that 1 exists in two tautomeric forms in solution (Figure 1). The structure of these tautomers were assigned tentatively as triketo form 1a and enol form 1b with the former predominating in approximately a 2:1 ratio. The proton-coupled ^{13}C spectrum



1a

1b

confirmed these assignments; that is, the resonance at 88.0 ppm of the vinyl carbon (C-5) in 1b was unsplit, whereas the signal at 49.1 ppm of the methine carbon (C-5) in 1a appeared as a doublet, and the methylene carbons in 1a and 1b were split into triplets. The methylene groups were identified by their signal intensities and association with the other two peaks. The proposed structure of 1b was confirmed further by comparison of its ^{13}C spectrum with that of the enolate anion of 5-(p-nitrobenzyl)barbituric acid sodium salt. Indeed, the orange solid 3

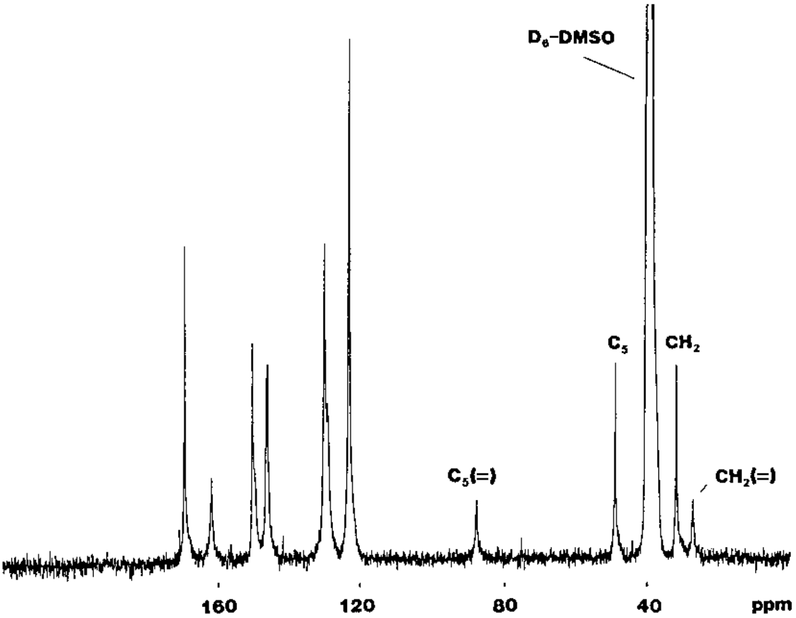


Figure 1. ^{1}H-decoupled ^{13}C spectrum of 5-(p-nitrobenzyl)barbituric acid (1) in hexadeuteriomethylsulfoxide (200 ppm). All the lines are broadened due to the keto-enol exchange equilibria.

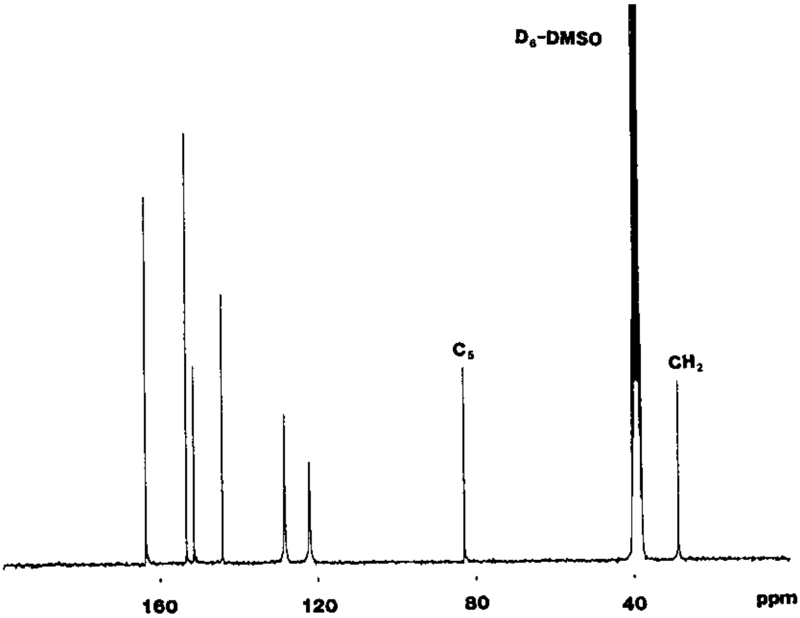


Figure 2. ^{1}H-decoupled ^{13}C spectrum of sodium 5-(p-nitrobenzyl)barbiturate (3) in hexadeuteriomethylsulfoxide (200 ppm). Note the sharpness of the lines as compared to Figure 1.

Table 1. ^{13}C Chemical shifts of some 5-substituted barbituric acids.[a]

Cmpd. No.	Solvent	Molecular Formula	Chemical Shifts (ppm)[b,c]									
			C_2	C_4	C_5	C_6	$-CH_2-$	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	Other
1a	DMSO	$C_{11}H_9N_3O_5$	150.8	169.6	49.1	169.6	32.0	d	130.2	123.4[e]	146.2	-
1b	DMSO	$C_{11}H_9N_3O_5$	150.2	162.2	87.9	162.2	27.3	d	129.4	123.3[e]	146.7	-
1a	DMSO/ 10% D_2O	$C_{11}H_9N_3O_5$	151.0	170.2	49.4	170.2	32.9	d	130.8	123.9[f]	146.7[f]	-
1b	DMSO/ 10% D_2O	$C_{11}H_9N_3O_5$	150.1	162.8	88.6	162.8	27.7	d	129.8	123.9[f]	146.8[f]	-
3	DMSO	$C_{11}H_8N_3O_5Na$	152.5	164.9	84.1	164.9	29.0	145.1	129.3	122.3	154.0	-
3	DMSO/ 10% D_2O	$C_{11}H_8N_3O_5Na$	153.1	165.7	86.4	165.7	29.2	146.0	130.0	123.8	153.7	-
3	1M NaOD	$C_{11}H_8N_3O_5Na$[g]	159.9	171.7	89.1	171.7	28.7	145.4	128.6	123.4	152.3	-
3	2M NaOD	$C_{11}H_8N_3O_5Na$[g]	159.2	170.8	88.0	170.8	27.6	144.1	127.4	122.2	151.2	-
9b[i]	DMSO	$C_4H_3N_3O_5$	149.9	159.6	112.9	159.6						

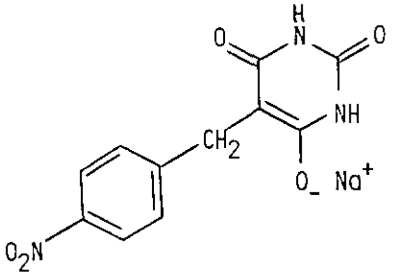
a. Numbering system used was such that ring N was assigned position one, carbon atom between the two ring nitrogens is C_2 and remainder of the atoms are numbered sequentially in clockwise direction. b. In δ (ppm) downfield from TMS and using solvent as double reference standard; d_6-DMSO = 39.5 ppm. c. All NaOD runs were recorded with external coaxial DMSO reference standard in a 5 mm o.d. nmr tube. d. Obscured by overlapping of signals. e. Two assignments could be reversed. f. Isomer chemical shifts tentatively assigned. g. Possibly a disodium salt. See the text for further discussion. h. Enolate anion of compound 11. i. At this concentration of NaOD two other species were formed which decomposed at higher concentration of NaOD.

Table 1. (Cont.)

Cmpd. No.	Solvent	Molecular Formula	Chemical Shifts (ppm)[b,c]									
			C_2	C_4	C_5	C_6	$-CH_2-$	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	Other
11a	DMSO	$C_{11}H_{10}N_2O_3$	150.5	169.9	49.3	169.9	33.4	137.4	127.3	126.7	d	-
11b	DMSO	$C_{11}H_{10}N_2O_3$	149.4	171.5	75.3	171.5	45.3	133.1	129.8	128.3	128.8	-
14[h]	2M NaOD[i]	$C_{11}H_9N_2O_3Na$	159.0	170.8	88.9	170.8	27.1	142.1	127.0	126.6	124.0	-
14[h]	6M NaOD	$C_{11}H_9N_2O_3Na$	159.8	171.1	88.3	171.1	27.1	142.2	126.9	126.5	123.9	-
12a[j]	DMSO	$C_{12}H_9N_3O_3$	150.6	169.6	49.1	169.6	32.5	144.2	132.1	130.0	118.9	109.5(CN)
12b[j]	DMSO	$C_{12}H_9N_3O_3$	150.2	162.0	87.9	162.0	27.4	147.4	129.2	128.8	d	108.6(CN)
2a[k]	DMSO	$C_{10}H_8N_2O_3$	151.1	169.3	55.1	169.3	-	134.6	129.3	128.9	128.0	-
2b[k]	DMSO	$C_{10}H_8N_2O_3$	150.0	161.3[e]	92.8	161.7[e]	-	134.8	131.9	131.1	126.6	-
15[k,l]	2M NaOD	$C_{10}H_7N_2O_3Na$	159.2	170.0	93.3	170.0	-	135.6	130.4	126.7	124.3	-
13a	DMSO	$C_4H_4N_2O_3$	151.7	167.8	40.0[m]	167.8						
13b	DMSO	$C_4H_4N_2O_3$	151.1	163.8	72[n]	163.8						
16[o]	2M NaOD	$C_4H_3N_2O_3Na$	160.6	172.9	110.6	172.9						

j. Treatment of compound 12 with base resulted in hydrolysis of cyano substituent. k. Data taken from ref. 4. l. Enolate anion of compound 2. m. Resonance obscured by solvent peak but readily observed in another solvent. n. Estimated value. o. Enolate anion of compound 13.

had almost identical ^{13}C absorptions (Figure 2), baring solvent effects, in d_6-DMSO as compound 1 in 1.0 and 2.0 molar NaOD (Table 1).

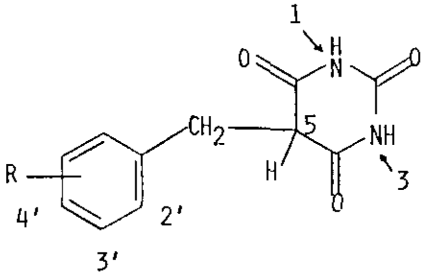


3

Proton-coupled and decoupled ^{13}C spectra of 3 assisted in identifying the aromatic resonances of 1a and 1b. Thus, the protonated carbons $C_{2'(6')}$ and $C_{3'(5')}$ of 3 were easily identified by their large $^{1}J_{CH}$ couplings (162.7 and 167.7 Hz, respectively) and by their ^{13}C chemical shifts.[5,6] The substituted carbons, $C_{1'}$ and $C_{4'}$, appeared as narrow triplets due to the long-range allylic coupling to benzenoid protons ($^{3}J\ C_{2'(6')}H_{3'(5')}$ = 10.5 Hz, $^{3}J\ C_{3'(5')}H_{2'(6')}$ = 6.1 Hz) at 145.1 and 154.0 ppm, respectively. Carbonyl carbon atoms were assigned by analogy with other studies,[1,7-13] signal intensity (C_4 and C_6 were of approximately equal intensity and twice the size of C_2) and by relaxation times (decreasing relaxation delay influenced signal intensity of C_2 to a greater extent than that of C_4 and C_6).

To study the role of the methylene group in the enol/keto equilibirum of 1, H/D exchange reactions were carried out with NaOD/D_2O in DMSO and monitored by ^{13}C nmr spectroscopy. In the initial experiment, a 10% deuterium oxide solution of 1 in d_6-DMSO gave a proton-decoupled ^{13}C spectrum which had a narrow triplet for the resonance at 49.3 ppm (C_5-D of keto form; $^{1}J_{CD}$ = 9.6 Hz), yet narrower triplet for the C_5 resonance of the enol at 88.9 ppm ($^{2}J\ C_5CHD$ = 4.0 Hz) and showed broadening of both methylene signals. This spectrum is consistent with hydrogen-deuterium exchange occurring not only at C-5 but also at the $-CH_2-$ group. Complete deuteration of the methylene group (as well as C-5) was accomplished by treating 1 with 2.0M NaOD/D_2O for 48 h and subsequent neutralization with conc D_2SO_4. Deuterium incorporation occurred also at the ring nitrogens as evidence by the disappearance of their signals in the ^1H nmr spectra (See Table 2).

Table 2. ^1H nmr chemical shifts of some selected 5-substituted barbituric acid derviatives (ppm)[a]

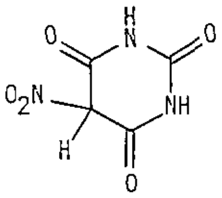


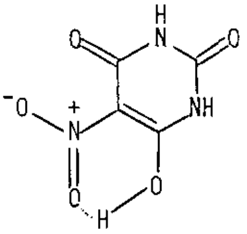
Cmpd. No.	Solvent	R	CH_2	H_5	NH	OH	$H_{2'}$	$H_{3'}$	$H_{4'}$
			Chemical Shifts (δ ppm)[b,c]				Aromatic		
1a	DMSO	4'-NO_2	3.7	4.1	11.3(2H)		7.42(2H,d)	8.11(2H,d)	
1b (enol)	DMSO	4'-NO_2	3.4		10.8(2H)	11.6	7.8 (2H,d)	8.5 (2H,d)	
1a	DMSO/ 10% D_2O	4'-NO_2	3.6	4.3	d		7.34(2H,d)	8.01(2H,d)	
1b (enol)	DMSO/ 10% D_2O	4'-NO_2	3.3		d	d	7.5 (2H,d)	8.2 (2H,d)	
3	DMSO	4'-NO_2	3.49		9.38(2H)		7.46(2H,d)	8.02(2H,d)	
3	DMSO/ 10% D_2O	4'-NO_2	3.48		d	d	7.41(2H,d)	7.98(2H,d)	
11a	DMSO	-	3.3	3.9	11.23(2H)		7.24(2H,m)		7.09(3H,m)
11b (enol)	DMSO	-	3.13		11.3 (2H)	10.8	e	e	e
12a	DMSO	4'-CN	3.7	4.1	11.3 (2H)		7.37(2H,d)	7.59(2H,d)	
12b (enol)	DMSO	4'-CN	3.3		10.7 (2H)	11.5	7.4 (2H,d)	7.9 (2H,d)	
2a	DMSO	5-phenyl	-	4.82	11.4 (2H)		7.32(2H,s)		7.29(3H,s)
2b (enol)	DMSO	5-phenyl	-		10.9 (2H)	11.6	e	e	e

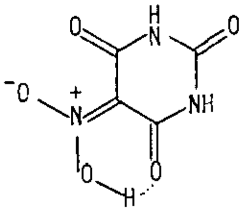
a. Recorded in δ (ppm) downfield from TMS and using a solvent as a double reference standard; d_6-DMSO impurity at 2.49 ppm. b. Resonances broadened due to exchange equilibria are reported to within ±0.1 ppm. c. Notation used was s, singlet; d, doublet; m, multiplet. d. Exchange in D_2O. e. Burried due to overlapping of signals in the aromatic region.

A possible mechanism to account for the H/D exchange of 1 is shown in Scheme 1. For convenience, only the H/D exchange between one of the methylene hydrogen atoms and the hydrogen atom at C-5 are shown. Accordingly, 1 reacts with base to yield initially the stabilized enolate anion having resonance structures 3a and 3b. Deuteration at C-5 by D_2O affords the 5-deutero compound, 4. However, OD^- or the enolate oxygen anion may also abstract a methylene hydrogen atom via a cyclic 5-membered transition state to give benzylic anion, 5a, which is resonance stabilized by the p-nitrophenyl group. Subsequent reaction of 5 with D_2O yields the methylene deuterated compound, 6. The reactions repeat until complete deuterium incorporation occurs, 7,8.

There is sufficient precedence in literature to warrant the existence of 5 as a viable intermediate. 5-Nitrobarbituric acid (9) was shown to exist in the solid state in the enol form 9b when anhydrous[14] and in the aci-nitro form 9c as a trihydrate.[15] The analysis of ^{13}C spectrum of 9 in DMSO solution (Figure 3) reveals that there are three carbon signals at 159.6 ($C_{4,6}$), 149.9 (C_2), and 112.9 ppm (C_5). This rules out tautomer 9a and suggests that 9b or 9c are present in solution. We have been unable to distinguish between the two since


9a

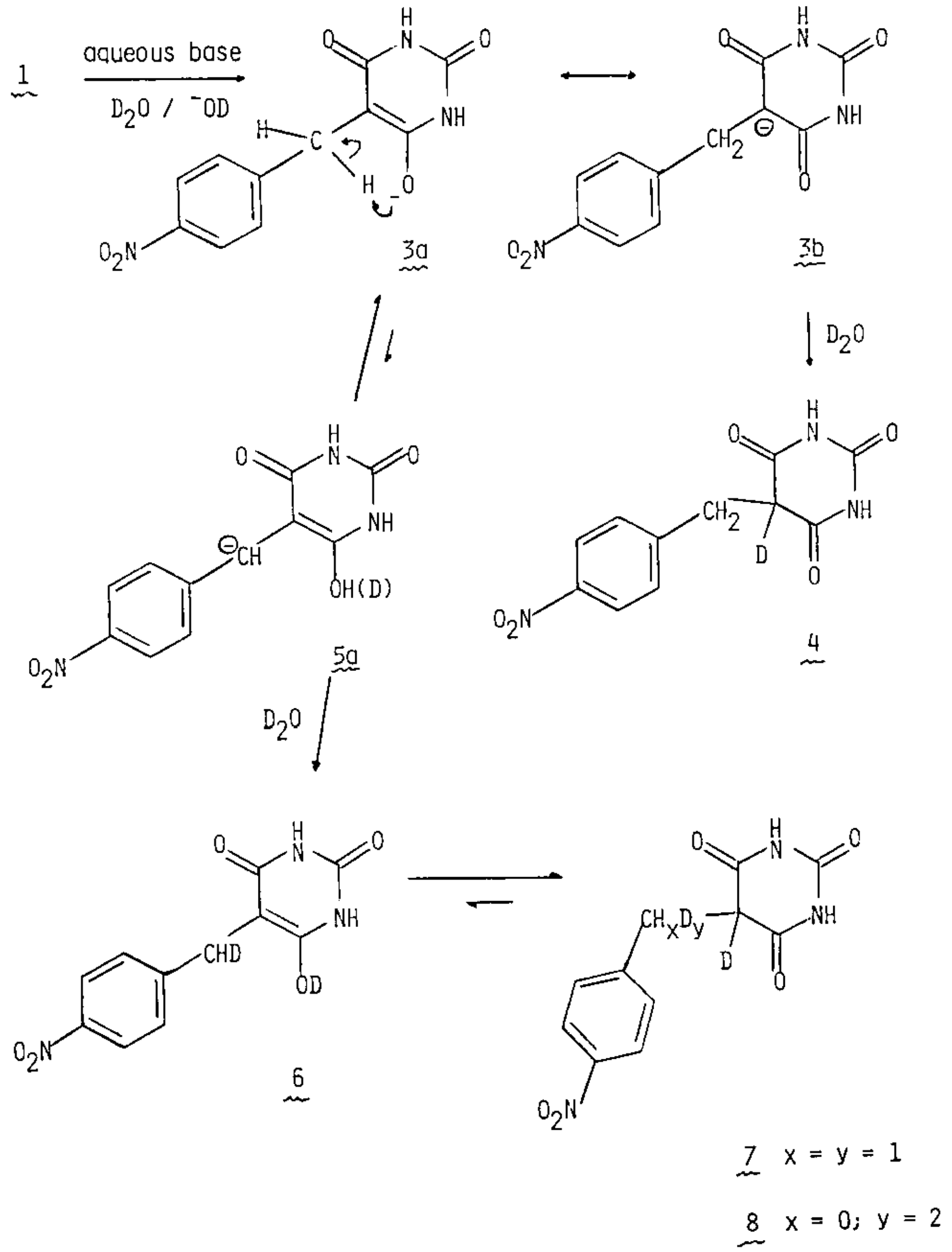

9b


9c

both should be stable and would have extensive hydrogen bonding. Molecular orbital calculations indicate that nitro-hydroxy structure 9b is the most stable.[16] Compound 9 formed a bright yellow sodium salt, 10, when treated with base as compared to the deep reddish-orange color of 3. The latter is probably due to the extended conjugation through the benzene ring. Because of the low solubility of 10 we could not obtain its ^{13}C spectrum in D_2O or at varying concentrations of base.

Finally, we tested the generality of the exchange principles on systems similar to 1. Table 3 shows the tautomeric isomer distribution in DMSO of 5-benzyl-

Scheme 1



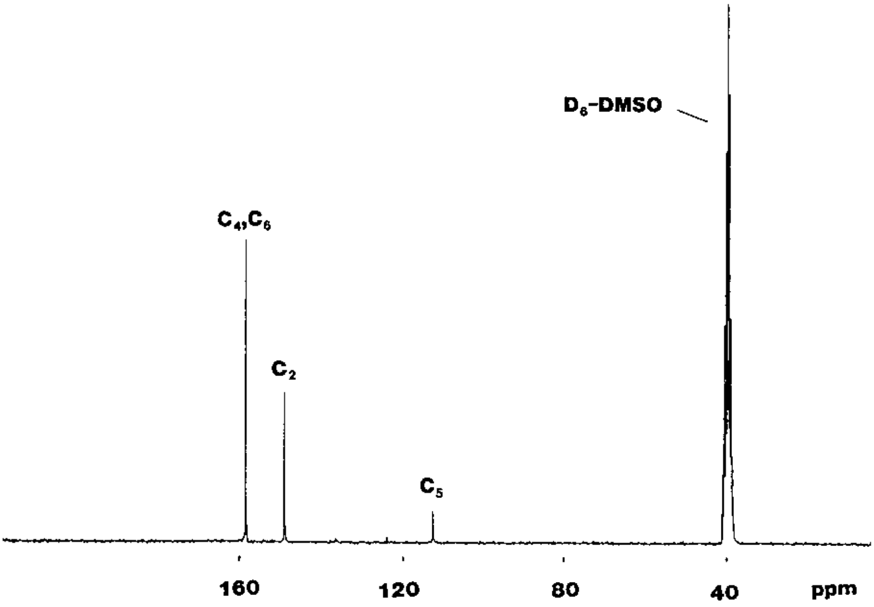


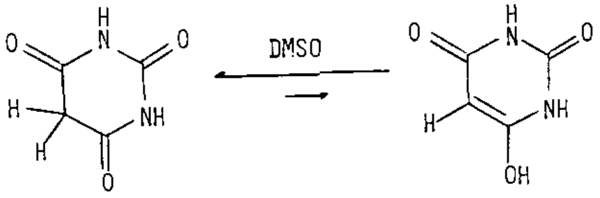
Figure 3. ^{1}H-decoupled ^{13}C spectrum of 5-nitrobarbituric acid, 9, in d_6-DMSO (200 ppm).

Table 3. Tautomeric isomer distribution of some barbituric acid derivatives in DMSO.[a]

Cmpd. No.	Substituent	Tautomer keto	Tautomer enol
1	5-(p-nitrobenzyl)	68% (1a)	32% (1b)
12	5-(p-cyanobenzyl)	83% (12a)	17% (12b)
11	5-benzyl	60% (11a)	40% (11b)
2	5-phenyl	76% (2a)	24% (2b)
13	none (barbituric acid)	99% (13a)	1% (13b)
9	5-nitro	none	100%[b]

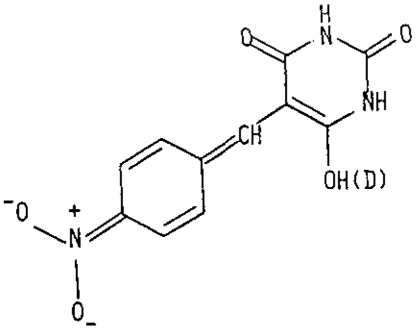
a. Data were derived from ^{13}C measurements and compared well (±5%) with distribution ratios calculated from ^{1}H nmr spectra. It is also noteworthy to point out that these values were very similar (±2-5%) to intensity ratios obtained from inverse-grated decoupling experiments (See Experimental). b. Hydroxy-nitro enol form 9b or aci-nitro form 9c as referred to in the text.

barbituric acid (11) 5-(*p*-cyanobenzyl)barbituric acid (12) 5-phenylbarbituric acid (2) and barbituric acid itself (13). We were surprised to find that even barbituric acid had 1% contribution from the enol form 13b in DMSO, contrary to two previous reports[17,18] which observed no enolization in DMSO. Tautomer 13b was not observed either by ^{1}H line shape analysis[18] or in any subsequent ^{13}C studies by other researchers.[7-13] This seemed inconsistent with evidence from UV studies which postulated tautomeric equilibrium between the keto and enol forms in water.[20,21] The activation energy for formation of lactim forms was reported to be very high and MO calculations show that resonance stabilization due to the extended conjugation is only 0.5 kcal/mole.[18,19] However, this type of stabilization must be very important in 5-phenyl compound, 2, in barbiturates with strong electron withdrawing group (i.e. 5-nitro derivative 9) and to some extent in 1 as far as stabilizing the anionic charge on the methylene group (as in 5b).



13a 13b

99 % 1 %



5b

EXPERIMENTAL

Starting Materials. Barbituric acid was commercially available. All other compounds were available from a previous study.[22]

Nmr Spectra. The ^{13}C nmr spectra in Table I were determined as 1 M solutions in d_6-DMSO with solvent peak as a reference ($\delta = 39.5$ ppm) unless indicated otherwise. ^{13}C spectra in sodium deuteroxide were recorded as 0.5 M solutions in a given concentration of NaOD contained in a 10 mm OD Wilmad nmr tube with 5 mm coaxial tube containing d_6-DMSO as an external reference standard. In all cases, deuterium resonance of the solvent was used as the internal lock signal. The spectra were obtained on a WP 200-SY Bruker spectrometer operating in a Fourier transform mode at the frequency of 200.130 MHz for proton and 50.327 MHz for ^{13}C nuclei. The spectrometer was interfaced with Winchester 24 MFD data system and was equipped with PTS 160 frequency synthesizer. The spectra were recorded at ambient temperature (35°C) with following spectral parameters: data set = 2500 scans for enolate anions, 3000-5000 for tautomeric mixtures for proton-noise decoupled spectra and 2-3 times as many counts for ^{1}H-coupled ^{13}C spectra; pulse width 14μs (45° flip angle); interpulse delay = 2-4 seconds, typically 4.0 seconds; sweep width = 10 KHz line broadening = 0.3 Hz; acquisition time = 0.5407 seconds; data size = 16K output data points (8K real). Expanded spectra with smaller spectral widths (2.5 KHz; 0.3 Hz resolution) were used for evaluation of spin-spin coupling constants.

Proton nmr spectra in Table 2 were run in same solvents with TMS external standard and using the solvent peak as a double reference. Because of limited solubility of all barbiturates studied, d_6-DMSO was used as the common solvent. Inverse-gated heteronuclear sequence experiments were carried out using the above-mentioned ^{13}C parameters and interpulse delays of 6, 8 and 10 seconds. No significant changes in relative peak areas of the tautomers were observed.

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