

THE STRUCTURE OF BARBITURIC ACID AND ITS 5,5-DIETHYL DERIVATIVE
AS REVEALED BY ^{15}N NUCLEAR MAGNETIC RESONANCE TECHNIQUES

Hans-Dieter Franken and Heinz Ruterjans*

Institute of Biophysical Chemistry, Johann Wolfgang Goethe University,
Theodor-Stern-Kai 7, D-6000 Frankfurt a.M. 70, F.R.G.

Franz Muller

Department of Toxicology, SANDOZ AGRO Ltd., CH-4002 Basel, Switzerland

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SUMMARY

The pH-dependent behavior of barbituric acid and its 5,5-diethyl derivative (veronal) has been investigated by ^{15}N -NMR spectroscopy using ^{15}N -enriched compounds. The pK values determined from the pH dependence of the ^{15}N chemical shifts are 3.87 and 12.46 for barbituric acid, and 7.89 and 13.3 for veronal. Owing to the symmetry of the molecule, the two nitrogen atoms show only one ^{15}N signal. A one-bond ^{15}N - ^1H coupling is observed only in a very limited pH range for barbituric acid and not at all for veronal in aqueous solution.

Separate ^{15}N signals are observed for the neutral and the monoanionic forms of barbituric acid. From temperature-dependence studies it is concluded that the deprotonation of the methylene group, i.e., the transition from sp^3 to sp^2 of the C(5) atom, requires high activation energy. The enthalpy of deprotonation is positive. It is shown that the monoanion of barbituric acid exists in the triketo form.

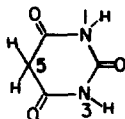
Several ^{13}C - ^{13}C , ^{13}C - ^1H , and ^{13}C - ^{15}N coupling constants are presented. These results indicate that the nitrogen atoms possess sp^2 character. In addition, the C(2)-N(1,3) bonds exhibit sp^2 character in the pH range of 2 to 10, whereas the C(4,6)-N(1,3) bonds show decreased sp^2 hybridization.

INTRODUCTION

Barbituric acid and its derivatives have been the subject of many spectroscopic studies in the past decades. It has been shown by UV spectroscopy^{1,2} and was confirmed later by X-ray studies³⁻⁵ that the trioxo form of barbituric acid is predominant in aqueous solution.^{1,2} These data were complemented by a variety of physical methods, including IR-,^{1,6,7} ORD-,⁸ CD-,⁹ proton-,^{7,10,11} and ^{13}C -NMR,¹²⁻¹⁵ as well as ^{14}N -nuclear-quadrupole resonance spectroscopy.¹⁶ In addition, some ^{13}C - ^{13}C and ^{13}C - ^1H coupling constants have been determined in ^{13}C -enriched barbituric acid derivatives.¹⁷ ^{15}N chemical shifts of various barbituric acid derivatives were also reported,¹⁸ and the ^{15}N chemical shifts of heterocyclic compounds have been reviewed.¹⁹

The series of NMR studies reported in this paper was carried out by the application of ^{15}N -NMR spectroscopy. The advantage of this method is the high sensitivity of the ^{15}N resonances to electronic and environmental effects. We investigated the pH-dependent behavior of

barbituric acid and its 5,5-diethyl derivative (veronal). The results obtained, in combination with published proton- and ^{13}C -NMR data, allow us now to describe the structure of barbituric acid in rather great detail.



EXPERIMENTAL SECTION

(1,3- $^{15}\text{N}_2$)barbituric acid and its 5,5-diethyl derivative were synthesized according to the modified method published elsewhere.²⁰ The starting compounds were (1,3- $^{15}\text{N}_2$)urea, enriched in ^{15}N to 96% in both nitrogen atoms (Rohstoff Einfuhr, Dusseldorf, F.R.G.), and 1,3-diethyl malonate and its 2,2-diethyl derivative. (4,5,6- $^{13}\text{C}_3$)- and (2,4,5- $^{13}\text{C}_3$)barbituric acids were synthesized by the same procedure, using (1,2,3- $^{13}\text{C}_3$)-1,3-diethyl malonate, and (1,3- $^{13}\text{C}_2$)-1,3-diethyl malonate and (^{13}C)-urea, respectively. The ^{13}C -enriched malonates and urea were from the Prochem Company, U.K. The compounds were enriched in ^{13}C to about 90%.

The NMR measurements were performed with 2 ml samples of a 0.1 M solution. To remove traces of possible paramagnetic impurities, the solutions were treated with activated Chelex 100 (Biorad, Richmond, VA, USA) prior to use.

The pH values were measured directly in the 10 mm NMR sample tubes (Wilmad Glass Co., Buena, NJ, USA), using a special electrode (Type 405-M-3, Ingold, Frankfurt, F.R.G.) combined with a Radiometer pH-meter PHM 26 (Radiometer, Copenhagen, Denmark).

For pH-dependent measurements the pH of the solution was adjusted to the desired value by adding small amounts of a concentrated NaOH or HCl solution. The pH values stated are accurate within ± 0.05 pH units.

The ^{15}N -NMR spectra were obtained on a Bruker HFX90 NMR spectrometer operating at 9.12 MHz. The spectra were accumulated in the Fourier transfer mode using a deuterium lock device. The deuterium signal was provided by $^2\text{H}_2\text{O}$ in a coaxial capillary inside the 10 mm NMR sample tube. Spectra were obtained with a spectral width of 3012 Hz, with and without proton noise decoupling, using a 60° pulse and a 3 s pulse interval. The ^{15}N chemical shifts are given in ppm relative to the external standard $^{15}\text{NH}_4\text{NO}_3$ (4 M solution 95% enriched) in 2 M HNO_3 . The temperature was determined to be $26 \pm 1^\circ\text{C}$, except where stated otherwise, and was maintained with a Bruker temperature control unit BST 100/700.

The ^{13}C measurements were made on a Bruker CXP300 NMR spectrometer operating at 75.6 MHz. Broadband decoupling at 2.0 W, if any, was used. All spectra were recorded using 30° pulses and a repetition time of 0.8 s.

Titration curves were calculated and fitted, using the pH dependence of the chemical shift values (δ) or the coupling constants (J) as described previously²¹

The proton exchange rate ($1/\tau_e$) was calculated according to Gutowsky and coworkers²² in the case of a fast exchange reaction, and according to Grunwald and coworkers²³ and Putte and Anderson²⁴ in the case of a slow exchange reaction

RESULTS

To our knowledge, ^{15}N -labeled barbituric acid and derivatives thereof have not been studied by physical techniques, except for (1,3- $^{15}\text{N}_2$,2- ^{13}C)hexobarbital, which has recently been used as an internal standard in gas-chromatography mass-fragmentation techniques.²⁵ In addition, the ^{15}N chemical shifts of (1,3- $^{15}\text{N}_2$)barbituric acid at pH 2.0 and of various barbituric acid derivatives in $(\text{CD}_3)_2\text{SO}$ have been reported.^{18,26}

Typical ^{15}N -NMR spectra of (1,3- $^{15}\text{N}_2$)barbituric acid as dependent on the pH of the solution are shown in Fig. 1. The spectra in Fig. 1A were obtained with broadband proton decoupling, those in Fig. 1B, without proton decoupling. The pH dependence of the ^{15}N chemical shifts of barbituric acid and its 5,5-diethyl derivative (veronal) is shown in Fig. 2. The values of the chemical shifts for the different ionized species of both compounds and the pK values derived from curve-fitting procedures are given in Table I.

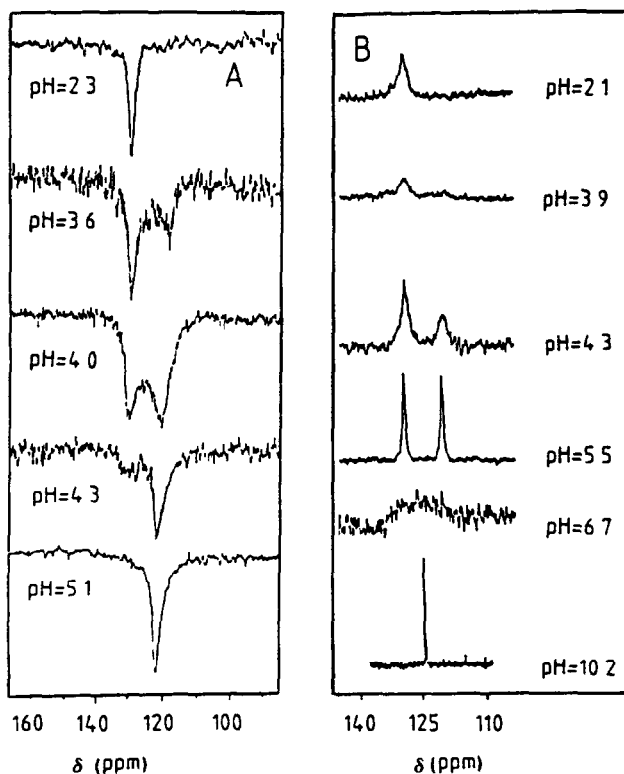


Fig 1. ^{15}N -NMR spectra of (1,3- $^{15}\text{N}_2$)barbituric acid as dependent on pH values at 26°C.

(A) Recorded under broadband proton decoupling conditions

(B) Recorded in the absence of broadband proton decoupling

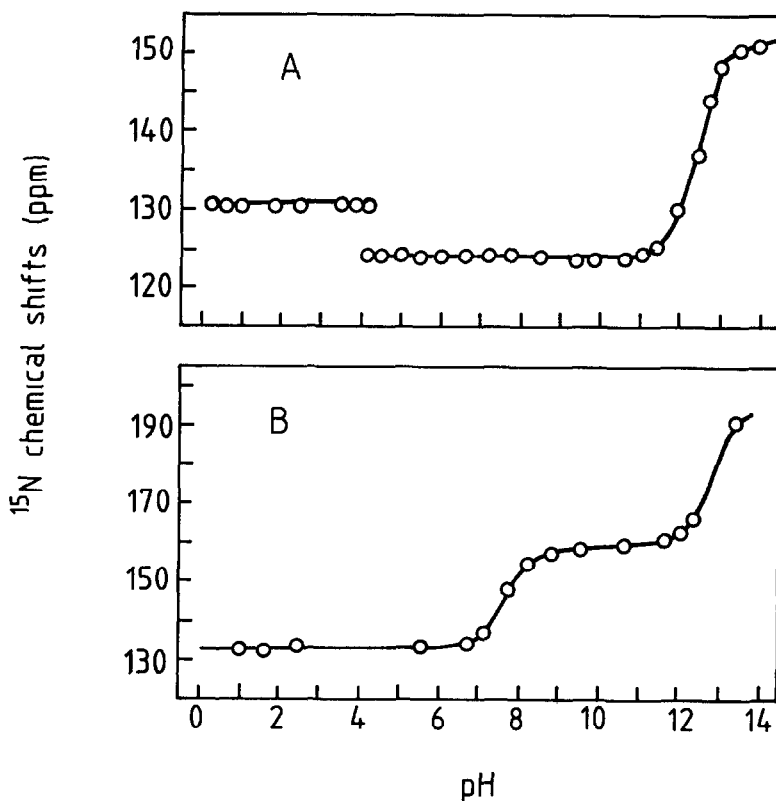


Fig. 2. The pH dependence of the ^{15}N chemical shifts of $(1,3-^{15}\text{N}_2)$ barbituric acid (A) and $(1,3-^{15}\text{N}_2)$ veronal (B) at 26°C. The open circles are experimental points. The theoretical curves (full lines) were calculated (see Experimental Section) using the following pK_a values: 3.87 and 12.46 for barbituric acid, and 7.89 and 13.3 for veronal.

Because of their symmetry, barbituric acid and veronal show only one ^{15}N resonance signal at 130.5 ppm and 133.5 ppm, respectively, for the two nitrogen atoms in the neutral state. However, deprotonation of the methylene group ($\text{pK}_1 = 3.87$) in barbituric acid causes the appearance of a second resonance line at 123.4 ppm in the spectrum (Fig. 1A). The intensity of this signal increases with increasing pH values while the signal at 130.5 ppm loses intensity. The signal at 123.4 ppm is therefore assigned to the two nitrogen atoms of the monoanion of barbituric acid. It is remarkable that the frequencies of the ^{15}N resonances remain constant throughout the deprotonation reaction. The pK_1 value of barbituric acid (Table I) was determined by plotting the ratio of the signal intensities of ionized and neutral barbituric acids against the pH.

Table I. ^{15}N chemical shifts of (1,3- $^{15}\text{N}_2$)barbituric acid and (1,3- $^{15}\text{N}_2$)veronal and the ionization constants of the two compounds as deduced from the pH dependence of the chemical shifts at 26°C.

Compound	Chemical shifts ppm ^a			pK Values	
	Neutral	Monoanion	Dianion	pK ₁	pK ₂
Barbituric acid	130.5 ± 0.1 ^b	123.4 ± 0.1 ^c	151.3 ± 0.2 ^d	3.87 ± 0.06	12.46 ± 0.02
Veronal	133.5 ± 0.1 ^e	161.5 ± 0.1 ^f	194.5 ± 0.3 ^g	7.89 ± 0.02	13.30 ± 0.20

^a The external reference used was 4 M $^{15}\text{NH}_4\text{NO}_3$ in 2 M HNO_3 .

^b At pH values below 3.0.

^c At pH values between 5.0 and 11.5.

^d At pH values above 13.5.

^e At pH values below 7.0.

^f At pH values between 9.0 and 11.5.

^g At pH values above 14.0.

Deprotonation of the imide group (pK = 12.46), in contrast to that of the methylene group, causes a downfield shift of +30 ppm of the ^{15}N resonance signal of barbituric acid (Fig. 2A). Similar shifts are observed for veronal upon formation of the monoanion and the dianion. Each deprotonation step leads to a downfield shift of about 30 ppm of the ^{15}N signal (Fig. 2B). Because of hydrolysis of veronal at high pH values, the pK value determined for the dianion is less accurate than the other values given in Table I.

The solvent-dependent ^{15}N chemical shifts of barbituric acid and veronal are given in Table II. Because of the much better solubility of veronal in organic solvents, this compound could be studied in a broader variety of solvents than barbituric acid.

The ^{13}C - ^{15}N and ^{15}N - ^1H coupling constants of barbituric acid and veronal have been determined in various solvents and are summarized in Table III. Where applicable the coupling constants were determined under broadband proton decoupling conditions.

The ^{15}N - ^1H coupling constant of about 93 Hz in barbituric acid was observable only in the limited range of pH 4 - 6, since the exchange rate of the imide protons is slow enough only in that range (see below). The coupling constant increases by about 7 Hz upon exchange of the aqueous solution for CF_3COOH . Above a pH value of 10.1 the ^{15}N signal is split into a doublet with a coupling constant of 1.6 Hz. This relatively small coupling could be observed owing to the very sharp resonance signal at this pH value (cf Fig. 1B). Under proton decoupling con-

Table II. Solvent dependence of the ^{15}N chemical shifts ^a of (1,3- $^{15}\text{N}_2$)barbituric acid and (1,3- $^{15}\text{N}_2$)veronal at 26°C.

Compound	Solvent	Chemical shift ppm
Barbituric acid	H ₂ O, pH 3	130.5
	CF ₃ COOH	127.9
Veronal	H ₂ O, pH 7	133.5
	dimethyl sulfoxide (DMSO)	133.6
	ethanol	132.4
	CF ₃ COOH	130.0
	CHCl ₃	130.4

^a See Table I.

ditions the doublet becomes a singlet. This coupling constant must be assigned to the three-bond coupling between the nitrogen atoms and the remaining proton on C(5) of ionized barbituric acid

The ^{13}C - ^{15}N one-bond coupling constants show a larger value for the $^{13}\text{C}(2)$ - $^{15}\text{N}(1,3)$ bonds than for the $^{13}\text{C}(4,6)$ - $^{15}\text{N}(1,3)$ bonds. The coupling constants exhibit pH dependence, they decrease considerably at pH values above the pK_a value of the deprotonation reaction of the imide groups. In contrast, the ionization of the methylene group in barbituric acid causes a slight increase of the $^{13}\text{C}(4,6)$ - $^{15}\text{N}(1,3)$ coupling constant.

Over the entire pH range studied, no ^{15}N - ^1H coupling could be observed for veronal in aqueous solution. The coupling constant determined for veronal in organic solvents differs only slightly from that of barbituric acid in aqueous solution (Table III). The coupling constant is somewhat influenced by the various organic solvents. The pH-dependent behavior of one-bond ^{13}C - ^{15}N couplings of veronal is similar to that observed for barbituric acid.

From an NMR study on flavins ²⁷ we had several ^{13}C -enriched barbituric acid derivatives at our disposal. The ^{13}C chemical shifts determined with these compounds in the present study (data not shown) are in fair agreement with published results.¹⁴ In Table IV the ^{13}C - ^{13}C and ^{13}C - ^1H coupling constants of barbituric acid are presented. We determined these coupling constants in order to complete the data published by others.¹⁷ The one-bond ^{13}C - ^{13}C and ^{13}C - ^1H coupling constants both show the same trend: they increase with the deprotonation of the methylene group and decrease with the ionization of the imide groups.

The two-bond ^{13}C - ^{13}C coupling constants apparently are little dependent on the pH of the solution.

Table III. Experimentally determined ^{15}N - ^1H and ^{13}C - ^{15}N coupling constants of (1,3- $^{15}\text{N}_2$)-barbituric acid and (1,3- $^{15}\text{N}_2$)veronal in aqueous and non-aqueous solvents at 26°C.

Compound	Solvent	Coupling	Coupling constant Hz
Barbituric acid	H ₂ O, pH 5.5	$^1J_{^{15}\text{N}(1,3)-^1\text{H}(1,3)}$	93.4
	CF ₃ COOH	"	100.1
	H ₂ O, pH 10.1	$^3J_{^{15}\text{N}(1,3)-^1\text{H}(5)}$	-1.6
	" pH 2.4	$^1J_{^{13}\text{C}(4,6)-^{15}\text{N}(1,3)}$	-10.8
	" pH 5.3	"	-10.2
	" pH 6.2	"	-11.7
	" pH 9.7	"	-11.9
	" pH 13.1	"	-8.9
	" pH 2.4	$^1J_{^{13}\text{C}(2)-^{15}\text{N}(1,3)}$	-19.7
	" pH 5.3	"	-19.1
	" pH 6.1	"	-19.8
	" pH 9.7	"	-19.0
	" pH 13.1	"	-4.9
	Veronal	acetone	$^1J_{^{15}\text{N}(1,3)-^1\text{H}(1,3)}$
ethanol		"	92.3
DMSO		"	91.8
CF ₃ COOH		"	93.7
CHCl ₃		"	93.5
H ₂ O, pH 1.5		$^1J_{^{13}\text{C}(4,6)-^{15}\text{N}(1,3)}$	-11.0
" pH 10.2		"	-7.4
" pH 1.5		$^1J_{^{13}\text{C}(2)-^{15}\text{N}(1,3)}$	-20.1
" pH 10.2		"	-6.8

The exchange rate of the imide protons with the water protons is dependent not only on the pH of the solution but also on its temperature. Fig. 3A shows the ^{15}N -NMR spectra of barbituric acid in aqueous solution at pH 5.5, as dependent on the temperature. With increasing temperature the linewidth of the two ^{15}N resonance signals increases and the intensity decreases, owing to an increase in the proton exchange rate. The coalescence point was found to be at 61°C. Similarly, increasing the temperature of a barbituric acid solution at pH 3.9 ($\text{p}K_1 = 3.87$) under broadband proton decoupling conditions, where both species exhibit a reso-

Table IV. ^{13}C - ^{13}C and ^{13}C - ^1H coupling constants of (4,5,6- $^{13}\text{C}_3$)- and (2,4,6- $^{13}\text{C}_3$)-barbituric acid in dependence on the pH value at 26°C.

pH	Coupling constants Hz		
	$^1\text{J}_{^{13}\text{C}(4,5)-^{13}\text{C}(6)}$	$^2\text{J}_{^{13}\text{C}(2)-\text{C}(4,6)}$	$^1\text{J}_{^{13}\text{C}(5)-\text{H}(5)}$
1.9	48.9	a	133.2
5.7	74.5	a	168.3
9.9	74.5	3.6	168.3
14.0	69.7	4.0	164.6

^a Not observed.

nance signal, results in coalescence of both signals at about 43°C, and the final resonance absorbs now at 126.1 ppm (Fig. 3B). It is interesting to note that with increasing temperature the intensity of the high-field signal initially increases more than that of the low-field signal. The temperature dependence of the chemical shifts of the two signals is presented schematically in Fig. 4.

The pH-dependent proton exchange rate was determined according to a published procedure.²¹ The results are shown in Fig. 5. In the range of fast exchange, the experimentally determined $^1\text{J}_{^{15}\text{N}(1,3)-^1\text{H}(1,3)}$ coupling constant of barbituric acid in aqueous solution at pH 5.5 (93.4 Hz) and that of veronal in CHCl_3 (93.5 Hz) have been used in the calculations.

The pH-dependent proton exchange rates are much smaller for barbituric acid than for veronal. These results are in agreement with the observation that no ^{15}N - ^1H coupling (see above) could be observed for veronal in aqueous solution. Both compounds show a similar pH-dependent behavior, except that the minimum of the exchange rate occurs at different pH values, namely at about pH 6 for barbituric acid and at about pH 3 for veronal.

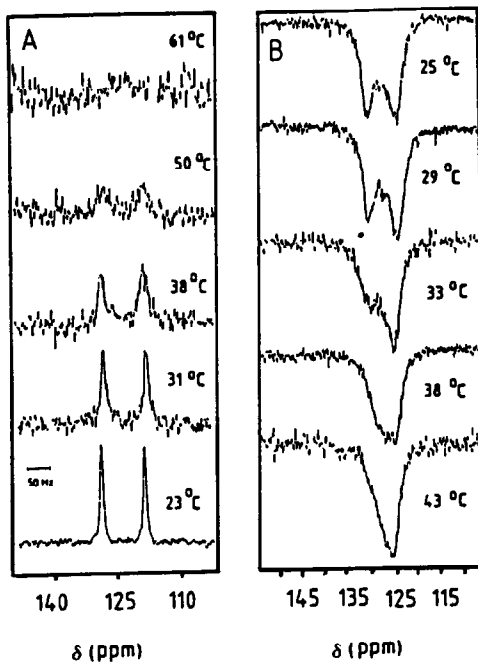


Fig. 3. Temperature dependence of the ^{15}N signals of $(1,3-^{15}\text{N}_2)$ barbituric acid.
 (A) Without broadband proton decoupling; pH = 5.5.
 (B) With broadband proton decoupling; pH = 3.9.

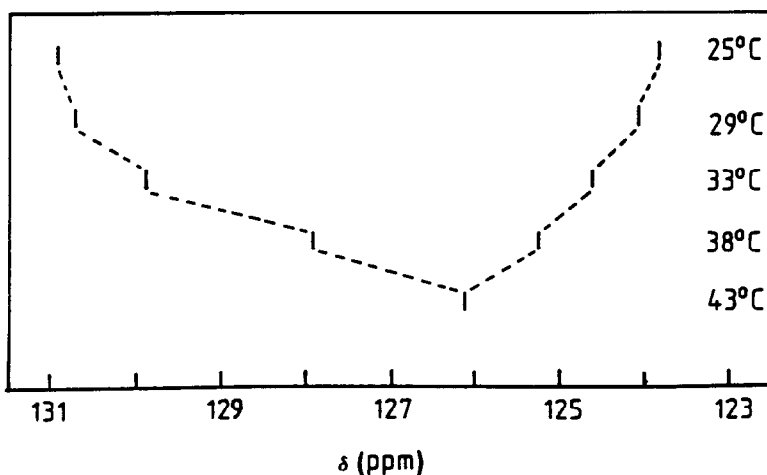


Fig. 4. Schematic representation of the temperature dependence of the ^{15}N chemical shifts of $(1,3-^{15}\text{N}_2)$ barbituric acid at pH 3.9.

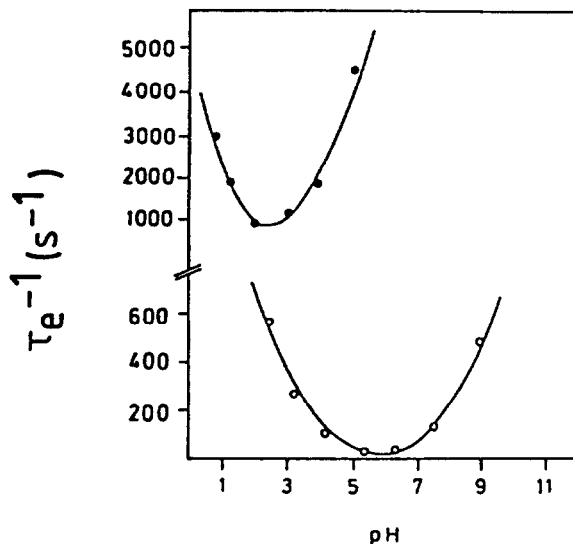


Fig. 5. The pH dependence of the proton exchange reactions of the imide protons of (1,3-¹⁵N₂)barbituric acid (o-o-) and (1,3-¹⁵N₂)veronal (●-●-) at 26°C.

DISCUSSION

¹⁵N chemical shifts yield valuable information on the structure of a molecule, in particular the shifts in dependence on the pH value of the solution, because the large range of chemical shifts of the ¹⁵N resonances makes it possible to detect even small perturbations in the electronic structure of a molecule.

The nitrogen atoms in heterocyclic compounds are commonly classified either as α -type (pyrrole type) or as β -type (pyridine type).²⁸ The theoretical background of the screening constant has been discussed in detail.²¹ At low pH values the nitrogen atoms of barbituric acid and veronal resonate at 130.5 ppm and 133.5 ppm, respectively. These values of the chemical shift indicate that the corresponding nitrogen atoms of the two compounds are both of the α -type.

Because of their symmetry, the N(1) and N(3) atoms of the neutral molecule of barbituric acid exhibit only one ¹⁵N resonance signal at 130.5 ppm. The intensity of this signal decreases with increasing pH values, and a new signal appears at 123.4 ppm in the spectrum. At the pK₁ value of barbituric acid (3.87, Table I) the two signals possess the same intensity. In the course of the deprotonation reaction both the protonated and the ionized species are present in the solution. The proton exchange reaction between the two species is usually fast with respect to the NMR time scale, leading to the observation of one averaged signal in the ¹⁵N-NMR spectrum. The observation of two separate signals in the course of the deprotonation reaction strongly suggests that the proton exchange reaction between the species is slow (see also below)

The frequency of the resonance signal is not affected in the pH range between 4 and 11. Further increase of the pH value of the solution leads to a drastic downfield shift of the resonance signal. The large downfield shift of about 30 ppm must be attributed to the ionization of one of the imide groups. This second ionization of barbituric acid and veronal decreases the π -electron density of the nitrogen atoms. This decrease, in turn, and the creation of a lone electron pair result in a decrease of the average excitation energy and thereby in a downfield shift of the ^{15}N resonances.

The pH dependence of the ^{15}N chemical shift of barbituric acid parallels that of the ^{13}C chemical shift of the C(4,6) atoms of barbituric acid.²⁹ Because of the high sensitivity of ^{15}N chemical shifts for protonation and deprotonation reactions, the pK_a values determined from the pH dependence of the ^{15}N chemical shift of barbituric acid are more accurate than published values determined by other techniques.^{2,29}

The ^{15}N resonance signal of veronal behaves in the same way as that of barbituric acid.

The ^{15}N chemical shifts of barbituric acid and veronal show a fairly strong solvent dependence (Table II). The chemical shift difference between the two extremes is about 3 ppm for both compounds. In CHCl_3 and CF_3COOH the ^{15}N chemical shifts are at high field and move downfield on changing from ethanol or dimethyl sulfoxide to H_2O . The downfield shifts are indicative of the formation of hydrogen bonds. The hydrogen bonds could occur with the nitrogen atoms as well as with the oxygen atoms of the molecules. The ^{13}C chemical shifts as dependent on the solvent could clarify this point, but such data are not available. However, as will be discussed below, the $^1\text{J}_{15\text{N}-1\text{H}}$ coupling constants also indicate that the nitrogen atoms are involved in the hydrogen bonds.

In the whole pH range studied, the exchange of the protons attached to the nitrogen atoms of veronal is so fast that no $^1\text{J}_{15\text{N}-1\text{H}}$ coupling is observed. The linewidths of the ^{15}N resonances with and without proton decoupling are identical and should represent the natural linewidth. The coupling constant in organic solvents is about 92 Hz (Table III). This indicates that the exchange rate of the protons in aqueous solution must be larger than 1000 s^{-1} , which is in agreement with the experimentally determined exchange rates (Fig. 5). The $^1\text{J}_{15\text{N}-1\text{H}}$ coupling for barbituric acid is observed only in the narrow range of pH 4 - 6. The coupling constant amounts to 93.4 Hz (Table III). This coupling constant increases considerably in CF_3COOH . Because of the limited solubility of barbituric acid in other organic solvents, it was not possible to further investigate the solvent dependence of the coupling constant. The coupling constants for veronal vary from 93.7 Hz in CF_3COOH and acetone to 91.8 Hz in DMSO. According to Binsch and coworkers,³⁰ the empirical equation

$$\% s = (0.43 \ ^1\text{J}_{15\text{N}-1\text{H}}) - 6, \quad (1)$$

relating the experimentally determined coupling constant to the hybridization of the corresponding binding orbitals, can be used to calculate the s character of the N-H bond, which is found to be between 33% and 37%. Since this value corresponds to an sp^2 hybridization of the nitrogen atoms, it can be concluded that barbituric acid and veronal possess a coplanar or al-

most coplanar structure with respect to the C(4)-N(3)-C(2)-N(1)-C(6) bonds of the molecules. This conclusion is in good agreement with crystallographic data.^{3,31}

Blomberg³² developed the empirical relationship:

$$r_{\text{NH}} = 3.12 - 0.0206 \ ^1J_{15\text{N}-1\text{H}}, \quad (2)$$

from which the distance between the nitrogen atom and the covalently attached proton can be estimated. Using this equation and the coupling constants given in Table III, N-H bond lengths of 0.105 to 0.122 nm can be calculated. These bond lengths correspond well with the theoretically expected value of 0.105 nm for an N-H bond.

The solvent dependence of the $^1J_{15\text{N}-1\text{H}}$ coupling constant strongly suggests hydrogen bond formation between the protons of the imide groups and certain solvents. CHCl_3 and CF_3COOH are not able to form hydrogen bonds with the group in question. In these solvents the coupling constant shows the largest value (Table III). Moreover, the ^{15}N chemical shift is upfield from that in the other solvents (Table II). The coupling constant decreases in barbituric acid in aqueous solution as compared to that in CF_3COOH . The same trend is observed for veronal on changing from ethanol to DMSO. The ^{15}N chemical shifts of both compounds undergo a downfield shift as the coupling constant decreases. As the strength of the hydrogen bond between the solute and the solvent increases, the $^1J_{15\text{N}-1\text{H}}$ coupling constant decreases owing to the increase of the N-H bond length. This process may be considered the initial step of a deprotonation reaction, which causes a downfield shift of the ^{15}N resonances. The combined results thus strongly indicate that the hydrogen bonds are formed with the imide, rather than with the carbonyl groups of barbituric acid and veronal. This conclusion is in agreement with the fact that DMSO forms a rather strong hydrogen bond, which cannot occur with a carbonyl group.

Usually the $^1J_{13\text{C}-15\text{N}}$ coupling constants are interpreted as being governed by various terms.³³ If α -type nitrogen atoms are involved in the C-N bond, as is the case with barbituric acid and veronal, the Fermi contact term is dominant and the simple empirical relation³⁰ between $^1J_{13\text{C}-15\text{N}}$ and the product of percent s character ($s_{13\text{C}}, s_{15\text{N}}$) in the corresponding bonding hybrids can be used.

$$80 \text{ J} = s_{\text{N}}s_{\text{C}}. \quad (3)$$

In Table III the $^1J_{13\text{C}(4,6)-15\text{N}(1,3)}$ and $^1J_{13\text{C}(2)-15\text{N}(1,3)}$ values for barbituric acid and veronal in dependence on the pH are given. Although we were not able to determine the sign of the $^1J_{13\text{C}-15\text{N}}$ coupling constants with our experimental device, it should be clear from the theoretical outline of Schulman and Venanzi³⁴ that these couplings between an α -type nitrogen and a carbon atom are negative. Other data also support this assumption.^{35,36}

The Binsch relation³⁰ (cf. eq. 3) predicts a $^1J_{13\text{C}-15\text{N}}$ value of about -15 Hz for the coupling between sp^2 -hybridized nitrogen and sp^2 -hybridized carbon atoms. The value of about -19 Hz for $^1J_{13\text{C}(2)-15\text{N}(1,3)}$ for both barbituric acid and veronal is in fair agreement with

the calculated value of -15 Hz, whereas the value of about -11 Hz for ${}^1J_{13\text{C}(4,6)-15\text{N}(1,3)}$ indicates a reduced s character of the C(4,6) hybrids.

Formation of the monoanion in barbituric acid does not significantly affect the 1J coupling constants. Deprotonation of one of the imide nitrogen atoms both in barbituric acid and in veronal, however, leads to a drastic decrease of the coupling constant, ${}^1J_{13\text{C}(2)-15\text{N}(1,3)}$ being much more affected than ${}^1J_{13\text{C}(4,6)-15\text{N}(1,3)}$.

At pH values above 10.1 one ${}^1J_{15\text{N}-1\text{H}}$ coupling of 1.6 Hz is observed. The 3J coupling constants are usually small if a lone pair exists at the nitrogen atom.³⁷ These coupling constants are known to be strongly dependent on the conformation of the bonds. The observed value roughly corresponds to that calculated for $\theta = 120^\circ$.³⁷

The ${}^1J_{13\text{C}(5)-1\text{H}(5)}$ coupling constant is about 133 Hz for neutral barbituric acid (Table IV). This value increases to about 168 Hz for the monoanionic molecule and decreases slightly upon formation of the dianionic species. It is known³⁸ that the one-bond ${}^{13}\text{C}-1\text{H}$ coupling is dependent on the s character of the carbon hybridization. The values of 133 Hz and 168 Hz correspond nicely with those expected for sp^3 and sp^2 hybridized carbon atoms, respectively. The values of the one-bond ${}^{13}\text{C}-1\text{H}$ coupling constants are thus in agreement with crystallographic data,³¹ showing that barbituric acid is almost coplanar in the monoanionic state and deviates from the planarity in the neutral state. These pH-dependent conformational changes occur at the C(5) position of the molecule.

The one-bond ${}^{13}\text{C}-{}^{13}\text{C}$ coupling constants (Table IV) show a pH dependence analogous to that of the one-bond ${}^{13}\text{C}-1\text{H}$ couplings. This observation is in excellent agreement with the fact that the one-bond ${}^{13}\text{C}-{}^{13}\text{C}$ coupling constants are also approximately correlated with the s character of the orbitals making up the bond.³⁸ The value of about 49 Hz observed in the neutral molecule corresponds well with that expected for an $\text{sp}^3\text{-sp}^2$ interaction, i.e., ± 50 Hz, and the value of about 75 Hz, observed for the monoanionic species, is in good agreement with that expected for an $\text{sp}^2\text{-sp}^2$ interaction, i.e., 70 - 76 Hz. The coupling constant decreases somewhat at pH values above 13. This observation can be ascribed to the ionization of the imide group, the negative charge on the nitrogen atoms leads to a decrease of the coupling constant where C(4) and C(6) are involved. Similar effects have been observed with ionized reduced flavin³⁹ and with amino acids upon ionization of the carboxyl group.⁴⁰ Long and Goldstein¹⁷ reported for a C(5)-dialkyl derivative of barbituric acid a one-bond ${}^{13}\text{C}-{}^{13}\text{C}$ coupling constant of about 48 Hz, this value is in agreement with our value for neutral barbituric acid. These authors observed also a decrease of the coupling constant at pH values above the $\text{p}K_1$ of the compound.

The two-bond ${}^{13}\text{C}-{}^{13}\text{C}$ coupling constant can be observed only at higher pH values (Table IV), apparently it is too small to be observed at low pH values. Since only a few 2J constants involving a central nitrogen atom have been reported,⁴¹ it is very difficult to interpret this coupling constant. Nevertheless, the coupling constants given in Table IV closely resemble those (3.2 Hz) reported by Long and Goldstein¹⁷ for 5,5-dialkyl barbituric acid. Hansen⁴¹ stated that these coupling constants are the sum of a positive two-bond coupling and a negative four-bond coupling.

The proton exchange rates of barbituric acid and veronal have been determined by methods extensively described previously.²¹ The pH-dependent proton exchange rate of barbituric acid (Fig. 5) shows a minimum at a pH value of about 6. The $^1J_{15N-1H}$ coupling (Fig. 1B) can be observed only in this pH range. At lower and higher pH values this coupling collapses owing to the increasing proton exchange rate. This increase can be explained by assuming an acid- and base-catalyzed exchange of the imide protons for protons of the solvent water. This proposal is further supported by the fact that lowering or increasing the pH of the solution leads to the collapse of the doublet without affecting the ^{15}N chemical shift. This mechanism is analogous to that of histidine.²¹

Neutral barbituric acid in solution has been proven to exist in the triketo form.^{1,2} In many exchange reactions the activation energy for a deprotonation reaction is small. This makes the existence of a tautomeric equilibrium at room temperature and in aqueous solution possible. As already mentioned above, this equilibrium cannot be determined in the case of a fast proton exchange reaction. The $^1J_{15N-1H}$ coupling in the monoanionic barbituric acid, however, is an excellent tool to investigate the possible existence of a tautomeric equilibrium in barbituric acid at pH 5.5 (Fig. 1B). At this pH value the proton exchange rate is low. If a tautomeric form were present in solution, a singlet resonance signal at lower field should be observed and, considering the intensity of the doublet at pH 5.5 (Fig. 1B), even a small amount of a tautomeric form would be detectable. This is, however, not the case. Increasing the temperature of the solution at pH 5.5 (Fig. 3A) results in the coalescence of the doublet to a singlet near 60°C. The chemical shift value remained unchanged in these experiments. This result confirms that in solution only the triketo form of the barbituric acid monoanion is present.

The observation of two signals at low pH values, i.e., for the neutral and the anionic species of barbituric acid, indicates that the deprotonation reaction of the C(5) methylene group is slow, and thus, that the activation energy is relatively high. The temperature dependence of the two ^{15}N NMR signals at pH 3.9 and under proton decoupling conditions is shown in Fig. 3B. The coalescence point at this pH value was observed near 48°C (Fig. 4). From this temperature dependence it is calculated that the activation energy necessary for the change of the hybridization state of the C(5) atom of barbituric acid from sp^3 to sp^2 is about 70 kJ/mol. This rather high energy requirement could be the reason why keto-enol tautomeric forms, involving the methylene group, are not observed in barbituric acid.

Finally, a few remarks should be made about an apparent inconsistency between the spectra of barbituric acid at pH 4.3, obtained with and without proton decoupling (Fig. 1A, B). At this pH value, the low-field signal belonging to the neutral species (130.5 ppm), in comparison with the high-field signal, is more intense without (B), than it is with (A), proton decoupling, although the opposite would be expected. This apparent contradiction results from the difference in the proton exchange rates of the neutral and the anionic species of barbituric acid. The ^{15}N signal of the anionic species is still split into a doublet at pH 4.3 (Fig. 1B), although the signal is broadened owing to the increased proton exchange rate. Since the difference between the values of the chemical shifts of the two species is about half that of

the one-bond ^{15}N - ^1H coupling constant, the low-field part of the signal of the anionic species overlaps that of the neutral species.

The results presented above demonstrate that ^{15}N -NMR spectroscopy contributes to a deeper insight into structural features of barbituric acid which are not otherwise accessible.

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