

## Protolytic equilibria of bromazepam

LIDIJA B. PFENDT<sup>1\*#</sup>, GORDANA V. POPOVIĆ<sup>2</sup>, TATJANA Ž. DAMJANOVIĆ<sup>1</sup> and  
DUŠAN M. SLADIĆ<sup>1#</sup>

<sup>1</sup>Faculty of Chemistry, University of Belgrade, Studentski trg 16, P. O. Box 158, YU-11000  
Belgrade and <sup>2</sup>Faculty of Pharmacy, University of Belgrade, P. O. Box 146, YU-11000 Belgrade,  
Yugoslavia

(Received 1 August, revised 30 October 2001)

The protolytic equilibria of bromazepam, an ampholyte sparingly soluble in water, in homogeneous and heterogeneous systems were studied in the pH range 0–14 at 25 °C and at ionic strength of 0.1 mol/dm<sup>3</sup> (NaCl). On the basis of <sup>13</sup>C-NMR spectra, the protonation site was predicted – in acidic media the pyridine nitrogen of bromazepam is protonated. The acidity constants of bromazepam were determined spectrophotometrically ( $pK_{a1}$  2.83 and  $pK_{a2}$  11.60) and potentiometrically ( $pK_{a1}$  2.99). In the heterogeneous system the following equilibrium constants were determined:  $K_{s0} = [HA]$  ( $pK_{s0}$  3.44),  $K_{s1} = [H_2A^+]/[H_3O^+]$  ( $pK_{s1}$  0.61), and  $K_{s2} = [A^-]/[H_3O^+]$  ( $pK_{s2}$  15.04).

*Keywords:* bromazepam, 1,4-benzodiazepines, acidity constants, heterogeneous equilibria.

### INTRODUCTION

Bromazepam (7-bromo-1,3-dihydro-5-(2'-pyridyl)-2H-1,4-benzodiazepin-2-one) belongs to the group of 1,4-benzodiazepines, compounds which are widely used as psychotropic drugs. Bromazepam has unique physico-chemical properties as the consequence of the pyridine moiety.

1,4-Benzodiazepines in aqueous solution may undergo simple acid-base reactions and hydrolytic degradation with changes in molecular structure.<sup>1–9</sup> The study of these reactions, within the physiological pH range, is of great importance because the absorption of these drugs in the gastrointestinal tract and transport through cell membranes is affected by the nature of the chemical species involved. Besides, identification of acidic and basic centers in molecules of pharmacologically active substances is significant in view of the structure – activity relationship.

Bromazepam has one deprotonation site (amide group) and two possible protonation sites (imine and pyridine nitrogens). There are some contradictions in previous

\* Corresponding author (E-mail: lpfendt@chem.bg.ac.yu.)

# Serbian Chemical Society active member.

papers<sup>10,11</sup> concerning the number of steps of the acid-base equilibrium reactions in the pH range 0–14 ( a two step reaction<sup>10</sup> with  $pK_{a1}$  3.5,  $pK_{a2}$  11.6, or a three step reaction<sup>11</sup> with  $pK_{a1}$  2.5,  $pK_{a2}$  5.2,  $pK_{a3}$  11.8) and about the exact protonation site. Therefore, the aims of this work were to locate the protonation site of bromazepam, to determine its acidity constants, and, as bromazepam is sparingly soluble in water, to study its solubility equilibria in a heterogeneous system and determine the appropriate equilibrium constants.

## EXPERIMENTAL

### *Apparatus*

A GBC 911A Spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Australia) with 1 cm silica cells was used for the spectrophotometric measurements. A PHM240 pH-meter (Radiometer) with a combined GK2401 B electrode (Radiometer) served for the determination of the pH values. Titrations were performed with a TTT-60 titrator with an ABU-12 autoburette (Radiometer).

<sup>13</sup>C-NMR spectra were recorded on a Varian/Gemini 200 instrument (at 50 MHz); chemical shifts ( $\delta$ ) are in ppm.

### *Reagents*

Bromazepam (Hofmann La Roche) of pharmaceutical purity grade was kindly provided by Krka (Novo Mesto, Slovenia). Stock solutions of bromazepam were prepared in ethanol (for spectrophotometric determinations) or in NaCl solution (for potentiometric determinations) by weighing accurately the dry substance. The solutions were stored at room temperature protected from light. Working solutions were prepared by dilution of the appropriate stock solution (the ethanol concentration was not higher than 1 %).

Other reagents: hydrochloric acid, sodium hydroxide, sodium chloride, sodium acetate, ethanol, dimethylsulphoxide-d<sup>6</sup>, and deuterotrifluoroacetic acid were of analytical reagent grade (Merck). All solutions were prepared with double distilled water.

The HCl and NaOH solutions were standardized potentiometrically. Acetate buffer pH 5.5 ( $c = 0.05 \text{ mol/dm}^3$ ) was prepared by mixing sodium acetate and HCl solutions in appropriate quantities.

### *Procedures*

*Determination of the protonation site.* <sup>13</sup>C-NMR spectra of bromazepam were recorded in d<sup>6</sup>-DMSO with and without an excess of CF<sub>3</sub>COOD. For comparison, <sup>13</sup>C-NMR spectra of nitrazepam were recorded under the same conditions. TMS was used as an internal standard.

*Determination of the acidity constants.* The acidity constants were determined spectrophotometrically ( $K_{a1}$  and  $K_{a2}$ ) and potentiometrically ( $K_{a1}$ ), at  $25.0 \pm 0.1$  °C at an ionic strength of  $0.1 \text{ mol/dm}^3$ , with a fast working procedure, in order to minimize errors due to hydrolytical changes in the molecular structure.

The UV spectra of bromazepam were recorded in  $1 \text{ mol/dm}^3$  HCl,  $0.1 \text{ mol/dm}^3$  NaCl (pH 6.8), and  $1 \text{ mol/dm}^3$  NaOH. For the determination of  $K_{a1}$  solutions of bromazepam ( $c_{Bz} = 4.950 \times 10^{-5} \text{ mol/dm}^3$ ) were prepared in  $p_{cH}$  range 2.05–3.68 with HCl solution and NaCl to reach  $I = 0.1 \text{ mol/dm}^3$ . For the determination of  $K_{a2}$  ( $c_{Bz} = 1.039 \times 10^{-4} \text{ mol/dm}^3$ ),  $p_{cH}$  range 10.88–11.78 was used and the  $p_{cH}$  adjusted with NaOH solution and NaCl to reach  $I = 0.1 \text{ mol/dm}^3$ . Two sets of experiments were performed.

The spectra were recorded over the range 220–400 nm at a scanning speed of 1000 nm/min against the corresponding blank. The absorbances were measured at 287 nm ( $K_{a1}$ ) and 350 nm ( $K_{a2}$ ).

For the potentiometric determination of  $K_{a1}$ ,  $20 \text{ cm}^3$  aliquots of stock solution of bromazepam ( $c_{Bz} = 3.327 \times 10^{-4} \text{ mol/dm}^3$ ) in  $0.1 \text{ mol/dm}^3$  NaCl were rapidly titrated with  $0.05 \text{ cm}^3$  increments of

standard HCl solution ( $c = 4.374 \times 10^{-4} \text{ mol/dm}^3$ ) till a pH of about 2.5 was reached. The total duration of the titrations was 3–4 min. The value of  $K_{a1}$  was found according to the formation function,  $\bar{n}$ , namely the mean number of protons bound to the base (bromazepam), with data from three times repeated experiments. The value of  $n$  was calculated according to the equation:

$$\bar{n} = \frac{c_{\text{HCl}} - [\text{H}_3\text{O}^+]}{c_{\text{Bz}}^{\text{tot}}} \quad (1)$$

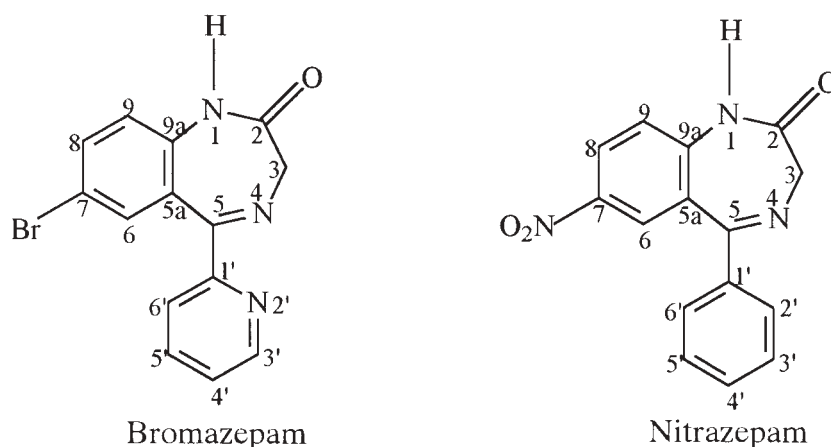
where  $c_{\text{HCl}}$  and  $c_{\text{Bz}}^{\text{tot}}$  are the concentrations of HCl and bromazepam in the titrated solution;  $[\text{H}_3\text{O}^+]$  was calculated from the measured pH values.

The measured pH values were converted into  $\text{p}c_{\text{H}}$  values according to relation:<sup>12</sup>  $\text{p}c_{\text{H}} = -\log [\text{H}_3\text{O}^+] = \text{pH} - A$ .  $A$  is a correction factor the value of which (0.12) was obtained by titrations of the standard HCl solution with the standard NaOH solution at  $25.0 \pm 0.1 \text{ }^\circ\text{C}$  and ionic strength  $0.1 \text{ mol/dm}^3$  (NaCl).

*Determination of the intrinsic solubility of bromazepam.* For the determination of the solubility of the molecular form of bromazepam ( $K_{s0}$ ), saturated solutions were prepared with an excess of bromazepam treated with  $0.1 \text{ mol/dm}^3$  NaCl. All the samples were thermostated at  $25.0 \pm 0.1 \text{ }^\circ\text{C}$  with occasional stirring until complete equilibration (4.5 h). The pH values of these heterogeneous systems were approximately 6.8. After equilibration, the mixtures were filtered, aliquots of  $1.5 \text{ cm}^3$  were transferred into  $25 \text{ cm}^3$  volumetric flasks and diluted to volume with  $0.1 \text{ mol/dm}^3$  NaCl. The actual concentration of the molecular form of bromazepam (HA) was determined spectrophotometrically at the wavelength of the absorption maximum ( $\lambda_{\text{max}} = 234 \text{ nm}$ ). Conformity with Beer's law had been previously verified.

## RESULTS AND DISCUSSION

The  $^{13}\text{C}$ -NMR spectral data (Table I) indicate that the pyridine nitrogen atom is the protonation site in the molecule of bromazepam. Significant changes of the chemical shifts of the pyridine carbons occurs upon protonation with  $\text{CF}_3\text{COOD}$  in  $\text{d}^6$ -DMSO. These changes are characteristic for protonation of the pyridine ring,<sup>13</sup> namely the signals corresponding to C-1' and C-3', vicinal to the nitrogen atom, are shifted upfield, while the signals corresponding to the more distant C-4', C-6', and especially C-5', are shifted downfield.



Scheme 1.

Additionally, the increase in chemical shift at C-5 upon protonation,<sup>12</sup> observed in the spectra of nitrazepam (Table I) and other 1,4-benzodiazepines with a benzene ring instead of a pyridine ring, due to deshielding of this carbon because of imine nitrogen protonation, is absent in the <sup>13</sup>C-NMR spectrum of protonated bromazepam. The upfield shift of the C-3 signal upon protonation of bromazepam is also much less pronounced than the corresponding shift in the spectra of nitrazepam (Table I) and other 1,4-benzodiazepines.<sup>12</sup> Based on the presented evidence, it can be concluded that the pyridine nitrogen is protonated. The fact that protonation of both basic nitrogen atoms in bromazepam does not occur, even in the presence of excess CF<sub>3</sub>COOD, can be explained by electron withdrawing effects of the protonated nitrogen atom and by stabilization of the mono-protonated form by some degree of intramolecular hydrogen bonding.

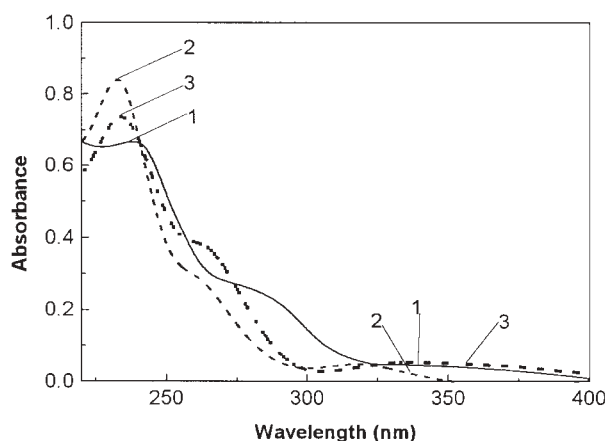


Fig. 1. Absorption spectra of bromazepam in solutions of different acidity: 1) 1 mol/dm<sup>3</sup> HCl (H<sub>2</sub>A<sup>+</sup> form); 2) 0.1 mol/dm<sup>3</sup> NaCl, pH 6.8 (HA form); 3) 1 mol/dm<sup>3</sup> NaOH (A<sup>-</sup> form);  $c_{Bz} = 2.514 \times 10^{-5}$  mol/dm<sup>3</sup>.

Most 1,4-benzodiazepines undergo hydrolytic degradation in aqueous medium. Therefore, the stability of bromazepam at 25 °C in solutions of different pH values in the range 0–14 was investigated spectrophotometrically in order to determine the optimal conditions for the determination of the acidity constants. In a solution with pH 12,

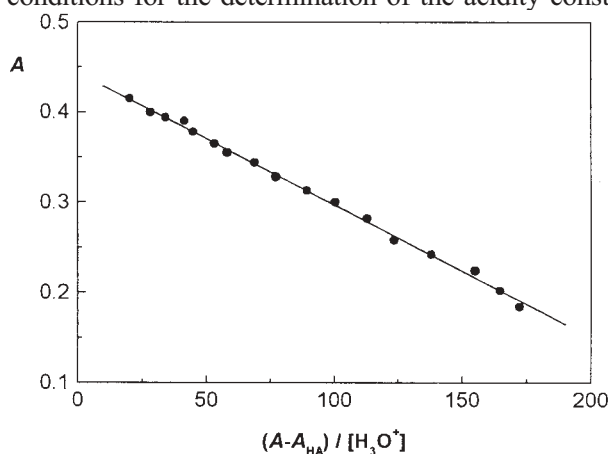


Fig. 2. Spectrophotometric determination of  $K_{a1}$  according to Eq. (2),  $c_{Bz} = 4.950 \times 10^{-5}$  mol/dm<sup>3</sup>,  $\lambda = 287$  nm.

changes in the spectra were observed after 1 h and in HCl solutions with pH 2 even after 10 minutes. No spectral changes were observed in 0.1 mol/dm<sup>3</sup> NaCl and in the pH region 6–10 even after 24 h. Therefore, a fast working procedure was used for the spectrophotometric and also for the potentiometric determination of the acidity constants, especially in acidic solutions. The absorption spectra of the different forms of bromazepam, which served for the determination of the optimal wavelengths for the acidity constant determinations are shown in Fig. 1. Both constants were determined according to the classical spectrophotometric equation<sup>14</sup> at 287 nm for  $K_{a1}$  and 350 nm for  $K_{a2}$ . The used wavelengths were chosen as the wavelengths where the relative ratio, not the difference, of the absorbances of the species is highest.

TABLE I. Chemical shifts (ppm) in the <sup>13</sup>C-NMR spectra of bromazepam and nitrazepam in d<sup>6</sup>-DMSO in the absence and in the presence of deuterotrifluoroacetic acid.

	Bromazepam		Nitrazepam	
	Bz	Bz + acid	Nz	Nz + acid
C - 2	170.2	169.6	170.2	168.7
C - 3	57.3	56.5	57.3	53.6
C - 5	167.9	167.2	168.7	175.0
C - 5a	127.7	125.5	126.6	124.0
C - 6	134.0	136.8 <sup>a</sup>	126.3	130.2
C - 7	114.3	115.8	141.7	142.9
C - 8	134.2	134.5 <sup>a</sup>	126.9	130.1
C - 9	123.4	124.4	122.5	122.9
C - 9a	139.1	140.6	145.3	146.8
C - 1'	156.1	151.1	138.6	134.3
C - 2'	–	–	129.7	132.5 <sup>c</sup>
C - 3'	148.7	142.0	128.7	129.7
C - 4'	125.2	128.1 <sup>b</sup>	131.0	134.8 <sup>c</sup>
C - 5'	137.4	147.7	128.7	129.7
C - 6'	123.7	127.7 <sup>b</sup>	129.7	132.5 <sup>c</sup>

<sup>a,b,c</sup> Assignments can be interchanged

As the molecule of bromazepam is rapidly degraded in acidic media,  $K_{a1}$  was also determined according to a transformed classical spectrophotometric equation and potentiometrically. The transformed spectrophotometric equation gives linear dependence:

$$A = A_{H_2A^+} - K_{a1} \frac{A - A_{HA}}{[H_3O^+]} \quad (2)$$

where  $A_{H_2A^+}$ ,  $A_{HA}$ , and  $A$  represent the absorbances of the protonated ( $H_2A^+$ ) and molecular (HA) form of bromazepam and their mixture at the specified wavelength, respectively.  $K_{a1}$  was calculated by linear regression analysis from the slope of corresponding line ( $pK_{a1}$  2.83) (Fig. 2), which is in good accordance with the value ob-

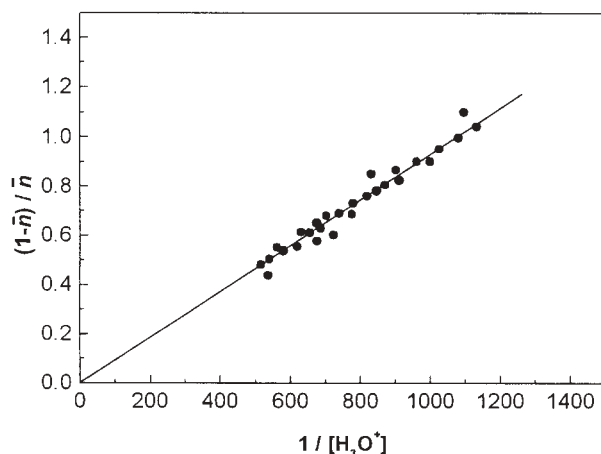


Fig. 3. Potentiometric determination of  $K_{a1}$  according to Eq. (3),  $c_{Bz} = 3.327 \times 10^{-4}$  mol/dm<sup>3</sup>.

tained according to the classical spectrophotometric equation ( $pK_{a1}$  2.89). Values for  $A_{H_2A^+}$  found by linear regression analysis (as the intercept with the ordinate,  $A_{H_2A^+} = 0.443$ ) and the measured value ( $A_{H_2A^+} = 0.434$ ) are also in good accordance.

The value of  $K_{a1}$  was determined potentiometrically from the slope of the corresponding line from three times repeated experiments according to the formation function<sup>15</sup> (Fig. 3):

$$\frac{1 - \bar{n}}{\bar{n}} = K_{a1} \frac{1}{[H_3O^+]} \quad (3)$$

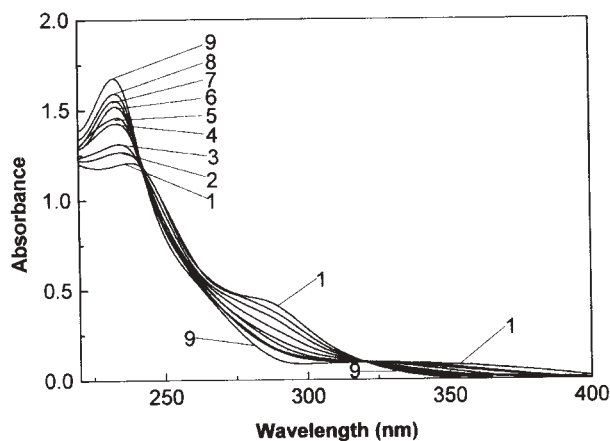


Fig. 4. Absorption spectra of bromazepam used for the determination of  $K_{a1}$ : 1) 1 mol/dm<sup>3</sup> HCl ( $H_2A^+$  form); 2)  $pC_H$  2.05; 3)  $pC_H$  2.39; 4)  $pC_H$  2.86; 5)  $pC_H$  3.05; 6)  $pC_H$  3.31; 7)  $pC_H$  3.62; 8)  $pC_H$  3.68; 9) acetate buffer  $pC_H$  5.50 (HA form);  $c_{Bz} = 4.950 \times 10^{-5}$  mol/dm<sup>3</sup>.

The potentiometric titration was not as precise as spectrophotometry, due to the longer time needed for the titration than for obtaining the spectra and to the low solubility of bromazepam in water ( $pK_{a1} < -\log c_{Bz}$ ). The appearance of clearly expressed isobestic points in the spectra used for the determination of the acidity constants (Fig. 4 and Fig. 5), demonstrates that a single acid-base pair occurs in both the studied equilib-

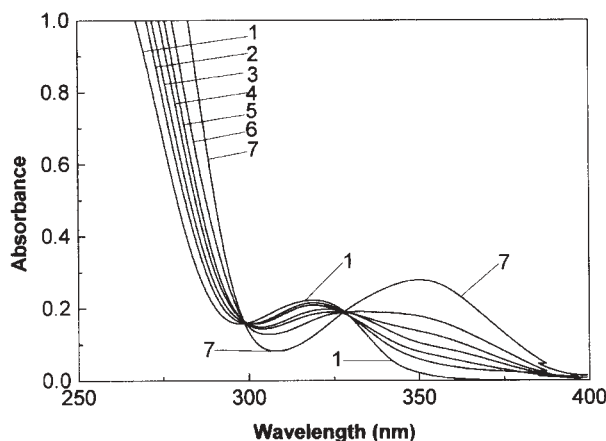


Fig. 5. Absorption spectra of bromazepam used for the determination of  $K_{a2}$ : 1)  $0.1 \text{ mol/dm}^3$  NaCl (HA form); 2)  $pC_H$  10.88; 3)  $pC_H$  11.07; 4)  $pC_H$  11.27; 5)  $pC_H$  11.47; 6)  $pC_H$  11.78; 7)  $1 \text{ mol/dm}^3$  NaOH ( $A^-$  form);  $c_{Bz} = 1.039 \times 10^{-4} \text{ mol/dm}^3$ .

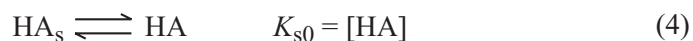
ria, *i.e.*, that the acid-base equilibria were not accompanied by any kind of secondary processes.

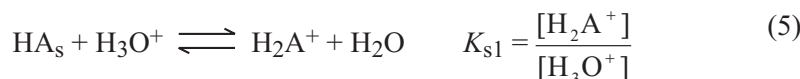
TABLE II. The concentration equilibrium constants ( $p\bar{K} \pm s$ ) in homogeneous and heterogeneous systems of bromazepam ( $I = 0.1 \text{ mol/dm}^3$ ;  $t = 25.0 \pm 0.1 \text{ }^\circ\text{C}$ )

Constant	Value	$n$	Equation
$pK_{a1}$	$2.89 \pm 0.03$	16	Classical spectrophotometric
	$2.83 \pm 0.01$	17	(2)
	$2.99 \pm 0.05$	28	(3)
$pK_{a2}$	$11.60 \pm 0.03$	12	Classical spectrophotometric
$pK_{s0}$	$3.44 \pm 0.02$	4	(9a)
$pK_{s1}$	$0.61 \pm 0.04$	–	(7)
$pK_{s2}$	$15.04 \pm 0.04$	–	(8)

The values of the determined acidity constants are listed in Table II. The  $pK_a$  value which corresponds to deprotonation of the amide group is in accordance with previous results.<sup>10,11</sup> However, the  $pK_a$  value of the protonated pyridine moiety differs greatly from the published results.<sup>10,11</sup> The procedure described in the literature<sup>10</sup> is not given with sufficient experimental details which are necessary for the study of a molecule like bromazepam that can undergo hydrolysis. Regarding the results given in Ref. 11, the  $pK_a$  value of 5.0 for the pyridine nitrogen in bromazepam, determined polarographically, seems unlikely, as there is an electron-withdrawing substituent in bromazepam, which should increase the acidity of the pyridine nitrogen. For instance, the  $pK_a$  of pyridine is 5.2<sup>16</sup> and  $pK_a$  values of 2,2'-bipyridine are  $pK_{a1}$  4.40<sup>17</sup> and  $pK_{a2}$   $-0.52$ .<sup>18</sup>

As other 1,4-benzodiazepines, bromazepam in its molecular form is sparingly soluble in water. Thus, the following equilibria between the solid phase ( $HA_s$ ) and solution in the heterogeneous system are possible:





From Eqs. (4)–(6) using terms for acidity constants it follows that:

$$K_{s1} = \frac{K_{s0}}{K_{a1}} \quad (7)$$

$$K_{s2} = K_{a2} K_{s0} \quad (8)$$

The equilibrium constants of bromazepam in heterogeneous system were determined by the solubility method. The concentration of bromazepam ( $c_{\text{Bz}}$ ) in a saturated unhydrolysed aqueous solution, *i.e.*, the solubility ( $S$ ), is given by the following expression:

$$c_{\text{Bz}} = [\text{H}_2\text{A}^+] + [\text{HA}] + [\text{A}^-] = S \quad (9)$$

Whitin the range  $\text{p}K_{a1} + 2 < \text{pH} < \text{p}K_{a2} - 2$ , the concentrations of  $\text{H}_2\text{A}^+$  and  $\text{A}^-$  could be neglected and Eq. (9) can be transformed into the following dependence:

$$c_{\text{Bz}} = [\text{HA}] = K_{s0} = S \quad (9a)$$

which represent the solubility of the molecular form of bromazepam. The solubility constant  $K_{s0}$ , *i.e.*,  $[\text{HA}]$ , was spectrophotometrically determined in 0.1 mol/dm<sup>3</sup> NaCl solution (pH 6.8). An experimental study of the heterogeneous equilibria in acidic and alkaline media was not possible because of the slow equilibration in the heterogeneous system and the fact that bromazepam undergoes relatively rapid hydrolysis. Therefore,  $K_{s1}$  and  $K_{s2}$  were calculated according to the determined constants  $K_{s0}$ ,  $K_{a1}$ , and  $K_{a2}$  and Eqs. (7) and (8). The equilibrium constants in heterogeneous system are listed in Table II.

From these data, the concentration of the unhydrolysed forms of bromazepam can be calculated as a function of  $\text{p}c_{\text{H}}$  according to following expression which was derived from Eqs. (4) – (9):

$$c_{\text{Bz}} = K_{s1} [\text{H}_3\text{O}^+] + K_{s0} + \frac{K_{s2}}{[\text{H}_3\text{O}^+]} = 10^{-(\text{p}K_{s1} + \text{p}c_{\text{H}})} + 10^{-\text{p}K_{s0}} + 10^{\text{p}c_{\text{H}} - \text{p}K_{s2}} \quad (10)$$

The minimal concentration of bromazepam in a saturated solution ( $\text{d}c_{\text{Bz}}/\text{d}c_{\text{H}} = 0$ ) is achieved when:

$$\text{p}c_{\text{H}} = \frac{\text{p}K_{s2} - \text{p}K_{s1}}{2} = \frac{\text{p}K_{a1} + \text{p}K_{a2}}{2} \quad (11)$$

and is  $3.64 \times 10^{-4}$  mol/dm<sup>3</sup> at  $\text{p}c_{\text{H}}$  7.22.

In solutions with  $\text{pH} < 5$  and  $\text{pH} > 10$ , bromazepam is not the only species present; there are also hydrolysis products, which is the subject of an on-going study.



*Acknowledgement:* The authors are grateful to the Research Fund of Serbia for partial financial support.

## ИЗВОД

## ПРОТОЛИТИЧКЕ РАВНОТЕЖЕ БРОМАЗЕПАМА

ЛИДИЈА Б. ПФЕНДТ<sup>1</sup>, ГОРДАНА В. ПОПОВИЋ<sup>2</sup>, ТАТЈАНА Ж. ДАМЈАНОВИЋ<sup>1</sup> и ДУШАН М. СЛАДИЋ<sup>1</sup>

<sup>1</sup>Хемијски факултет, Универзитет у Београду, Студенски брз 16, б. бр. 158, 11000 Београд и

<sup>2</sup>Фармацеутички факултет, Универзитет у Београду, б. бр. 146, 11000 Београд

Проучаване су протолитичке равнотеже бромазепама, амфолита из класе 1,4-бензодиазепина, слабо растворног у води. Испитивања су вршена у хомогеном и хетерогеном систему у рН интервалу 0–14, на температури 25 °С и при јонској сили 0,1 mol/dm<sup>3</sup> (NaCl). На основу <sup>13</sup>C-NMR спектра претпостављено је да до протоновања у молекулу бромазепама долази на пиридинском атому азота. Киселинске константе одређене су спектрофотометријски (pK<sub>a1</sub> 2,83 и pK<sub>a2</sub> 11,60) и потенциометријски (pK<sub>a1</sub> 2,99). У хетерогеном систему одређене су следеће равнотежне константе: K<sub>s0</sub> = [НА] (pK<sub>s0</sub> 3,44), K<sub>s1</sub> = [H<sub>2</sub>A] / [H<sub>3</sub>O<sup>+</sup>] (pK<sub>s1</sub> 0,61) и K<sub>s2</sub> = [A<sup>-</sup>][H<sub>3</sub>O<sup>+</sup>] (pK<sub>s2</sub> 15.04).

(Примљено 1. августа, ревидирано 30. октобра 2001)

## REFERENCES

1. W. W. Han, G. J. Yakatan, D. D. Maness, *J. Pharm. Sci.* **65** (1976) 1198
2. W. W. Han, G. J. Yakatan, D. D. Maness, *J. Pharm. Sci.* **66** (1977) 573
3. W. W. Han, G. J. Yakatan, D. D. Maness, *J. Pharm. Sci.* **66** (1977) 795
4. H. V. Maulding, J. P. Nazareno, J. E. Pearson, A. F. Michaelis, *J. Pharm. Sci.* **64** (1975) 278
5. W. F. Smyth, M. R. Smyth, J. A. Groves, S. B. Tan, *Analyst* **103** (1978) 497
6. W. F. Smyth, J. A. Groves, *Anal. Chim. Acta* **134** (1982) 227
7. N. Inotsume, M. Nakano, *J. Pharm. Sci.* **69** (1980) 1331
8. J. C. Vire, G. J. Patriarche, B. G. Hermosa, *Anal. Chim. Acta* **196** (1987) 205
9. L. B. Pfenndt, G. V. Popović, *J. Chem. Soc. Perkin Trans.* **2** (1994) 1845
10. S. Inui, M. Yamamoto, H. Nakae, S. Asada, *Yakuzai Gaku* **42** (1982) 71
11. M. R. Smyth, T. S. Beng, W. F. Smyth, *Anal. Chim. Acta* **92** (1977) 129
12. L. B. Pfenndt, D. M. Sladić, T. J. Janjić, G. V. Popović, *Analyst* **115** (1990) 383
13. H. O. Kalinowski, S. Berger, S. Braun, *<sup>13</sup>C-NMR-Spektroskopie*, Georg Thieme Verlag, Stuttgart, 1984, pp. 347, 363
14. A. Albert, E. P. Serjeant, *The Determination of Ionization Constants*, 2<sup>nd</sup> Ed., Chapman and Hall, London, 1971, p. 44
15. H. Rossoti, *The Study of Ionic Equilibria*, Longman, London, New York, 1978, p. 19
16. J. Bjerrum, *Acta Chem. Scand.* **18** (1964) 843
17. S. Cabani, G. Moretti, E. Scrocco, *J. Chem. Soc.* (1962) 88
18. F. H. Westheimer, O. T. Benfey, *J. Am. Chem. Soc.* **78** (1956) 5309.