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Solubility and acid–base behaviour of midazolam in media of different pH, studied by ultraviolet spectrophotometry with multicomponent software

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Abstract: The solubility of midazolam is <0.1 mg ml^{-1} at neutral pH and it increases considerably in acidic media. A pK_a value of 6.04 at 24 ± 1°C was calculated from the solubility data. In acidic media midazolam is reversibly converted to the corresponding benzophenone (open-ring form). The extent of this reaction was investigated by assaying the two compounds simultaneously by UV spectrophotometry with multicomponent software. The structure of the benzophenone derivative was verified by IR, MS and ^1H and ^{13}C NMR spectrometry.

Keywords: *Midazolam; hydrolysis; stability; UV spectrophotometry; benzophenone identification.*

Introduction

Midazolam, 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-*a*][1,4]benzodiazepine, is a basic drug of the benzodiazepine family. Its basicity is due to the nitrogen in the imidazole ring fused to the benzodiazepine skeleton. Some of the literature data on the solubility of midazolam are contradictory [1–3], and the pK_a values reported range from 5.50 to 6.20 [1, 4, 5]. Because the compound is basic, water-soluble salts such as maleates and lactates can be prepared.

Benzodiazepines are susceptible to hydrolysis in acidic media; hydrolysis follows apparent first-order kinetics. The seven-membered benzodiazepine ring is usually opened at the azomethine bond and an open-ring benzophenone is formed. In most cases (estazolam, etizolam, clotiazepam [6], diazepam [7], flunitrazepam, fludiazepam and flurazepam [8]), the benzophenone has been tentatively identified; spectrophotometric analysis of degraded solutions has been based on chloroform extraction of the parent drug, the open-ring benzophenone remaining in the aqueous layer. Scission of the 1,2-amide bond has been reported as well, in the hydrolysis of chlordiazepoxide [9, 10], nitrazepam [11, 12] and oxazepam [13], for example. In mildly acidic conditions hydrolysis of the benzodiazepine is reversible and the open-ring benzophenone is converted back to the original compound when the solution is made basic. In strongly acidic conditions all 1,4-benzodiazepinones degrade to aminobenzophenones.

Triazolo- and imidazo-benzodiazepines have a five-membered nitrogen-containing ring fused to the 1,2-positions in the benzodiazepine skeleton. This fused ring makes the molecule more stable. Thus, in the acid hydrolysis of estazolam [6], triazolam [14–17] and midazolam [4, 5, 15, 18] only the 4,5-azomethine bond is broken and an open-ring benzophenone is formed. Hydrolysis of these compounds likewise obeys first-order kinetics.

Konishi *et al.* [14] isolated the open-ring benzophenone of triazolam by preparative thin layer chromatography (TLC) and quantified it by gas–liquid chromatography (GLC). Jimenez *et al.* [17] later confirmed its identity by IR spectrometry and ^1H and ^{13}C NMR spectrometry after lyophilization of the acidic solution.

A few chromatographic and spectroscopic data are available for the open-ring benzophenone of midazolam [4, 18]. Bhattacharryya and Grant [18] assayed midazolam and the corresponding benzophenone simultaneously using ^1H and ^{19}F NMR spectrometry but no ^1H (or ^{13}C) NMR data were reported.

The aim of the present study was to confirm the identity of the open-ring benzophenone form of midazolam by spectrometric methods and to investigate the extent of the conversion from the closed- to the open-ring form in acidic

media using UV spectrophotometry with multicomponent software. The solubility of midazolam in media of different pH was determined and the pK_a value was calculated from the solubility data.

Experimental

Materials

Midazolam was kindly supplied by Hoffmann-La Roche (Basle, Switzerland). The identity and purity of the substance were verified by TLC and by UV, IR, ^1H NMR and ^{13}C NMR spectrometry. All other reagents and solvents were of analytical grade.

Apparatus

Melting points were determined with an electrothermal digital melting point apparatus and are uncorrected. The UV spectra were recorded using a Philips PU 8740 UV–vis spectrophotometer with multicomponent software (Kalman filter technique). The pH of the solutions was measured with a Radiometer PHM83 autocal pH meter. The solubility tests on midazolam were conducted in a 624 environmental incubator shaker. IR spectra were recorded on a Unicam SP3-200 instrument. The 200 MHz ^1H and ^{13}C NMR spectra were recorded on a Jeol JMN-FX 200 FT spectrometer using TMS as internal or external standard. The MS spectra were recorded on a Jeol JMS-SX 102 mass spectrometer with direct inlet (electron energy 70 eV).

Thin layer chromatography

TLC experiments were conducted on precoated 0.25-mm silica-gel aluminium 60 F_{254} plates; the solvent systems were toluene–2-propanol (7:3, v/v) (A) and chloroform–acetone–ethanol (3:1:1, v/v/v) (B); the length of run was 7.5 cm; and an unsaturated chamber was used. The spots were detected under UV light (254 nm).

Determination of the solubility

To a 100-ml Erlenmeyer flask were added 20 ml of a buffer solution and a sufficient amount of midazolam to allow maximum solubility of the midazolam at different pH values. Britton–Robinson buffers, prepared according to the method of Brczina and Zuman, were used in the pH range 3.32–9.50 [19]. The pH of the medium was measured before and after dissolution of the midazolam. Each solution (four parallel samples) was shaken in the shaker for 3 h at 150 rpm and 24 ± 1°C. The samples were filtered (Schleicher and Schüll, Spartan 30/B) and diluted to the appropriate volume. The amount of midazolam dissolved was determined spectrophotometrically at the absorption maximum (220–222 nm depending on the pH of the medium) by comparing the absorbance of the test solution with that of a known standard (3×10^{-5} M) in the same buffer.

Determination of the pK_a value of midazolam

The pK_a value was calculated from the solubility data of midazolam in the pH range 5.4–6.2 by the following equation [20]:

$$pK_a = pH + \log [(S_0'/S_1) - 1],$$

where S_1 = solubility of the neutral molecular species (0.055 mg ml^{-1}) and S_0' = observed solubility.

Determination of the amounts of the open- and closed-ring forms of midazolam at different pH values

UV spectrophotometry with multicomponent software was used to determine the amounts of the open- and closed-ring forms of midazolam. A stock solution of midazolam (1 mg ml^{-1}) was prepared in ethanol; 1 ml of this solution was mixed with buffer, 0.1 M HCl, 1 M HCl or 0.1 M NaOH to obtain a solution containing 1 mg/100 ml of midazolam (final ethanol concentration, 1%). The standard spectrum for the closed-ring form was recorded in buffer at pH 7.04 (mean of three determinations) and the standard spectrum for the open-ring form was recorded in 1 M HCl (mean of three determinations). Buffers of pH 1.70–7.04 and 0.1 M HCl were used in the multicomponent assay. The number of parallel samples was four; for each sample six determinations were performed. All the solutions were allowed to stand for 2 h before analysis to allow equilibrium to be achieved.

To study the effect of ionic strength on the acid–base equilibria, different amounts of potassium chloride were added to buffer solutions of pH 1.80 and the pH of the solutions was adjusted with 1 M HCl to pH 1.70. The ionic strengths of the resulting solutions were 0.21, 0.37, 0.52 and 0.68 M. Four parallel samples were prepared of each solution and each sample was analysed six times.

Preparation and characterization of the dihydrochloride salt of the open-ring form of midazolam: 5-aminomethyl-1-[4-chloro-2-(2'-fluorobenzoyl)phenyl]-2-methyl-imidazole dihydrochloride (II)

The compound was prepared according to the method of Walser *et al.* [5]. White crystals from 2-propanol, m.p. 296–300°C (decomp.). UVλ_{max} (1 M HCl) 260 nm (ϵ = 12 000 l mol^{-1} cm^{-1}), UVλ_{max} (EtOH) 202.5 nm (ϵ = 31500 l mol^{-1} cm^{-1}), 222 nm (ϵ = 33800 l mol^{-1} cm^{-1}). IR (KBr disc): 3200–2200 (broad band, primary amine, NH_3^+), 1650 (C=O), 1610, 1520, 1480, 1460, 1300, 1215, 830, 760 cm^{-1}. TLC R_f 0.55 (A), R_f 0.75 (B). ^{1}H NMR (DCl, 20%, TMS in $CDCl_3$ external standard): 7.86–6.88 (m, 8H, aromatic), 3.92 (s, 2H, CH_2), 2.15 (s, 3H, CH_3) ppm. Some ^{1}H NMR data in DMSO-d_6 (TMS internal standard) are included in Table 1. ^{13}C NMR (DCl, 20%, TMS in $CDCl_3$ external standard): 192.2 (C=O), 161.1 (C-2', J_{CF} = 255.0 Hz), 148.0–120.2 (aromatic and imidazole ring C:s and CH:s), 117.4 (C-3', J_{CF} = 22.0 Hz), 33.3 (CH_2), 11.7 (CH_3) ppm. MS *m/z* (% rel.int.): 345 (M + 2, 20), 343 (M, 56), 328 (23), 327 (22), 325 (58), 312 (55), 311 (33), 310 (100), 297 (18), 222 (17), 163 (12), 123 (22), 109 (12), 95 (13), 36 (20).

Table 1
^{1}H NMR spectral data for midazolam (I) and the corresponding open-ring benzophenone (II)

	CH_3	CH_2
I in $CDCl_3$	2.56	4.05 (d, J_{HH} = 13.0 Hz) 5.13 (d, J_{HH} = 13.0 Hz)
I in DCl (20%)	2.64	4.60 (d, J_{HH} = 15.2 Hz) 5.23 (d, J_{HH} = 15.2 Hz)
I in DMSO-d_6	2.53	4.22 (d, J_{HH} = 13.2 Hz) 5.24 (d, J_{HH} = 13.7 Hz)
II in DCl (20%)	2.15	3.92 (s)
II in DMSO-d_6	2.43	2.82 (s)

Characterization of midazolam (I)

White powder, m.p. 164°C. UVλ_{max} (EtOH) 200 nm (ϵ = 36400 l mol^{-1} cm^{-1}). IR (KBr disc): 3030 (aromatic CH), 3000–2850 (aliphatic CH), 1615 (imine), 1490, 1455, 1420, 1310, 1215, 825, 770, 750 cm^{-1}. TLC R_f 0.55 (A), R_f 0.75 (B). ^{1}H NMR ($CDCl_3$, TMS internal standard): 7.67–6.93 (m, 8H, aromatic), 5.13 (d, 1H, CH_2, AB system, $J_{H,H}$ = 13.0 Hz), 4.05 (d, 1H, CH_2, AB system, $J_{H,H}$ = 13.0 Hz), 2.56 (s, 3H, CH_3) ppm. Some ^{1}H NMR data in 20% DCl (TMS in $CDCl_3$ external standard) and in DMSO-d_6 (TMS internal standard) are included in Table 1. ^{13}C NMR ($CDCl_3$, TMS internal standard): 164.3 (C-6), 160.3 (C-2', J_{CF} = 250.6 Hz), 144.1–124.0 (aromatic and imidazole ring C:s and CH:s), 116.2 (C-3', J_{CF} = 25.0 Hz), 46.1 (CH_2), 15.0 (CH_3) ppm. MS *m/z* (% rel.int.): 327 (M + 2, 12), 325 (M, 35), 312 (36), 310 (100), 297 (12), 163 (14), 75 (10), 44 (20), 39 (13).

Results and Discussion

The solubility of midazolam in buffers of different pH is shown in Table 2. The solubility of the molecular form of midazolam in the pH range 7.9–9.5 is 0.055 mg ml^{-1} (RSD 1.1%), which is in good accord with the value given by the manufacturer (in water <0.1 mg ml^{-1}). The solubility of midazolam is dramatically increased in acidic media because of the ionization of the molecule.

Table 2
Solubility of midazolam in buffers of different pH

pH after dissolution of midazolam	Solubility mg ml^{-1} ± RSD% (n = 4)	pK_a
9.50	0.054 ± 2.6	
8.57	0.055 ± 11.5	
7.95	0.055 ± 3.4	
6.39	0.082 ± 0.9	6.08
6.22	0.093 ± 5.6	6.06
6.04	0.115 ± 5.3	6.08
5.84	0.142 ± 6.3	6.04
5.61	0.193 ± 5.6	6.01
5.43	0.247 ± 7.1	5.97
4.97	0.368 ± 9.6	
4.20	2.357 ± 3.0	

The mean of the pK_a values obtained was 6.04 (RSD 0.7%), which is in good agreement with the pK_a values reported in the literature: 6.15 by Walser *et al.* [5], 6.0 by Vire *et al.* [15] and 6.20 by Clarke's Handbook [1]. Some divergent data have also been reported (pK_a 5.50) [4].

The UV spectra of midazolam in media of four different pH values are shown in Fig. 1. The change in the spectrum is due to formation of the open-ring benzophenone form of midazolam (Fig. 2). The change is reversible; when the solution is made basic the spectrum of molecular midazolam appears. The spectra reported at different pH values show one isosbestic point (255 nm), which suggests a one-to-one transformation from **I** to **II**.

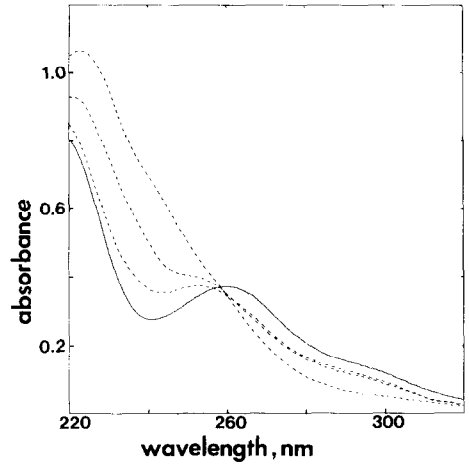

Figure 1
UV spectra of midazolam (3×10^{-5} M) in media of different pH:pH 7.04, pH 2.60, pH 2.11 and 1 M HCl. The absorbance at 220 nm decreases as the solution becomes more acidic.

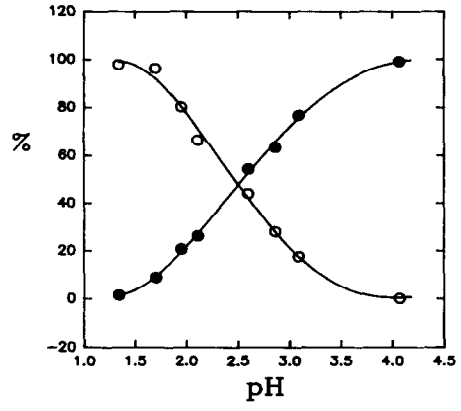

Figure 3
The proportions (in %) of midazolam (●) and the corresponding benzophenone (○) as a function of the pH of the solution.

The results of the multicomponent assay are shown in Fig. 3. At pH > 4 midazolam exists in the closed-ring form and at pH 1–4 an equilibrium between **I** and **II** is attained. The results are in good agreement with those reported by Bhattacharryya and Grant [18] using fluorine-19 NMR spectrometry. Their fluorescamine method gave slightly different results, probably because the pH of the medium had to be increased to 8 before complex formation would take place between the benzophenone and fluorescamine. In the multicomponent assay the results are very sensitive to changes in the wavelength range. Because it was impossible to prepare a mixture with known amounts of midazolam and the dihydrochloride salt of the benzophenone owing to the reversible change from one form to another, the range (220–320 nm) where the sum of compounds **I** and **II** was closest to 100% was chosen.

In the experiments at different ionic-strengths the proportion of the open-ring benzophenone was 86, 94, 96 and 93% at ionic strengths 0.21, 0.37, 0.52 and 0.68 M, respectively. The relative standard deviation of the values for the four samples at each ionic strength was 0.8–2.0%. One result (ionic strength 0.21) differed from the others, possibly because of a slight change in the pH of the medium. The results show that ionic strength has no effect on the equilibrium between compounds **I** and **II** in acidic media. The rate of hydrolysis, which reportedly is affected by ionic strength [e.g 9–11], was not investigated.

Elucidation of the structure of the open-ring benzophenone revealed a few differences from that of midazolam although the two compounds could not be separated by TLC. In the UV spectrum in EtOH, compound **II** showed

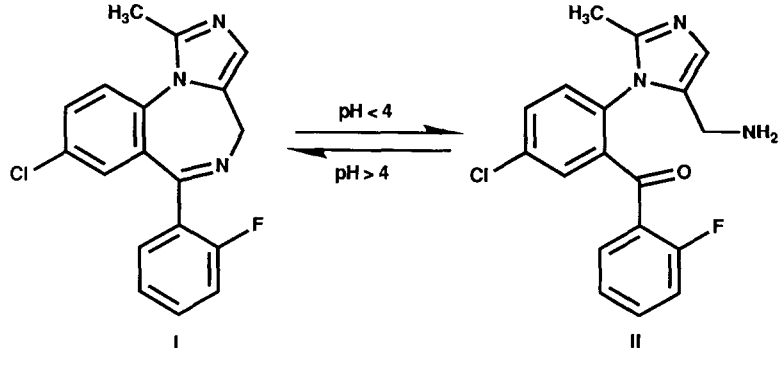

Figure 2
The structures of midazolam (**I**) and the corresponding open-ring benzophenone (**II**).

two maxima at 202.5 and 222 nm, whereas compound **I** showed a single maximum at 200 nm. In the IR spectrum, compound **II** showed a broad band in the 2200–3200 cm^{-1} region, whereas compound **I** absorbed weakly at 3030 and 3000–2850 cm^{-1}. The increase in the absorptions for compound **II** is due to the protonation of the primary amine. A second clear difference in the spectra was the carbonyl band of **II** at 1650 cm^{-1} which was lacking in the spectrum of **I**.

The MS spectrum of **II** showed a molecular peak at 343 *m/z* due to $C_{18}H_{15}ClFN_3O$. Chlorine satellite peaks were clearly visible after the molecular and base peaks, confirming the presence of one chlorine atom. The base peak at 310 *m/z* was the same as for midazolam and the fragmentation of **II** resembled that of **I**. The resemblance may indicate an actual similarity of the fragmentation patterns of **I** and **II**; alternatively the heating involved in the MS analysis may have converted **II** to **I**.

The ^1H and ^{13}C NMR spectra of compound **II** recorded in DCl showed a trace amount of compound **I**. The clearest differences in the ^1H NMR spectra of the two compounds were the absorptions due to CH_2 protons. In the spectrum of compound **I** these protons formed an AB system of two doublets, whereas in the spectrum of compound **II** there was only one singlet peak (Table 1). In the ^{13}C NMR spectrum of the benzophenone derivative the resonance of the CH_2 carbon was shifted upfield compared with the resonance of the corresponding carbon in midazolam; this shift was due to the opening of the seven-membered ring. In the spectra of both compounds the fluorine-substituted phenyl ring gives rise to the characteristic couplings with carbon and fluorine atoms [21]. In DMSO-d_6 compounds **I** and **II** existed in a 1:1 equilibrium, although only compound **II** was added to the solvent.

On the basis of the spectral results, the structure of compound **II** was confirmed to be that in Fig. 2.

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