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An examination of acid-base equilibria of 1,4-benzodiazepines by spectrophotometry

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Changes of ultraviolet absorption spectra with pH in solution were used to determine pKa values for six 1,4-benzodiazepines. Diazepam, chlordiazepoxide and medazepam as a result of protonation of the molecule in acidic solutions, were found to each have one pKa, while two pKa values were observed for oxazepam, nitrazepam and lorazepam, because of protonation in acid and deprotonation of the neutral molecule in alkaline media. The spectra are explained by considering them to be superimposed spectra of the two benzene rings, one mono-substituted and one tri-substituted, within the molecule. Sites of protonation (principally at nitrogen atoms in position 4 in the diazepine ring) and deprotonation (for oxazepam, nitrazepam and lorazepam) are predicted and the differences in the observed pKa values explained.

Knowledge of the structure of drug species in media of physiologically relevant pH is of value in determining the degree to which they are absorbed by body organs and how they permeate cell membranes. In addition to the pharmacological value of such information, data are obtained which are necessary for the determination of those pH ranges best used for the extraction of the drugs and their metabolites from body fluids.

Ultraviolet spectrophotometry has been used for the determination of the 1,4-benzodiazepines, a group of therapeutically important hypnotic tranquillizers, in formulations (National Formulary, 1970) and after extraction of the drugs and their metabolites from body fluids (Lafargue, 1970; De Silva, Koechlin & Bader, 1966; Beyer & Sadee, 1969; De Silva & Strojny, 1971). To the end mentioned above, pKa values of six 1,4-benzodiazepines (I-VI) have been determined by studying the variation of their ultraviolet spectra with pH. Predictions can be made about the structures of the species existing over the pH range 0–14 and the probable sites of protonation and deprotonation.

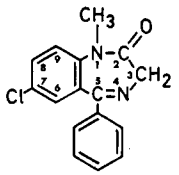
MATERIALS AND METHODS

Apparatus

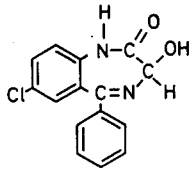
Spectra over the ultraviolet wavelength range were recorded in solutions maintained at 20° using a Perkin-Elmer double beam 137 ultraviolet visible spectrophotometer. Matched 1 cm path length silica cells were used and the instrument was flushed with dry nitrogen to eliminate stray radiation effects.

Reagents

Samples of the benzodiazepines were obtained from the sources mentioned in the acknowledgements.



I Diazepam



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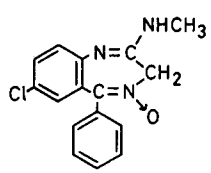
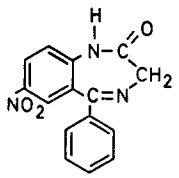
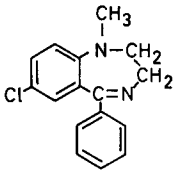


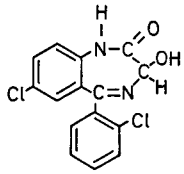
III Chlordiazepoxide



IV Nitrazepam



V Medazepam



VI Lorazepam

Stock solutions of each drug (about 10^{-3}M) were prepared in Analar methanol, and kept in the dark under refrigeration to minimize the possibility of decomposition.

A stock Britton-Robinson buffer solution (pH just over 2·0) composed of a mixture of boric acid, phosphoric acid and acetic acid all 0·04 M, was prepared using Analar grade reagents. From this, buffer solutions of varying pH were prepared by addition of 0·1M sodium hydroxide solution to the stock solution and measuring the pH on a meter.

Experimental techniques

Experimental solutions were prepared by diluting the appropriate amount of stock benzodiazepine solution with the appropriate buffer to give a drug concentration of 5×10^{-5}M. To extend the pH range studied at either end of the scale, M HCl, 0·1M HCl, 0·1M NaOH and M NaOH were used. The range from about pH 1 to 13 was scanned for each drug in increments of 1 pH unit to determine the approximate position of each pKa value by observation of spectral changes over the whole range. The region around each pKa value was then studied in more detail using buffers differing by increments of 0·3 pH units. From the spectra obtained, pKa values were evaluated using the Henderson equation (Davidson & Smyth, 1972).

The wavelength region scanned was from 200 to 390 nm. Slow scan speed (8 min for the range) was used, and the instrument reference beam contained a blank of buffer solution containing the same amount of methanol as the samples.

RESULTS

For the six compounds examined, three, diazepam, medazepam and chlordiazepoxide exhibited only one pKa vaue over the range, while oxazepam, nitrazepam and lorazepam each exhibited two pKa values (Table 1). Chlordiazepoxide and medazepam exhibit very similar behaviour, as do oxazepam and lorazepam. All six drugs could be shown to undergo protonation in acid, but only nitrazepam, oxazepam and lorazepam underwent deprotonation in alkaline media.

Spectral data for each drug are given in Table 1, H_2A^+, HA and A^- representing protonated, neutral and deprotonated species respectively*, while Figs 1 and 2 show

* Preliminary evidence as to this designation is given by 90–100% extraction of chlordiazepoxide (Kennett, 1972) and nitrazepam (Clifford, 1972) into dichloroethane at $pK_2 > pH > pK_1$.

Table 1. *pKa values and comparison of different forms of benzodiazepines (electronic spectra)*

Compound	pKa_1	pKa_2	H_2A^+ λ_{max}	H_2A^+ ϵ ($\times 10^4$)	HA λ_{max}	HA ϵ ($\times 10^4$)	A^- λ_{max}	A^- ϵ ($\times 10^4$)
Diazepam	3·3		241	2·8	231	3·3	—	—
			286	1·3	253(s)	1·7	—	—
Chlordiazepoxide	4·6		245	2·6	250(s)	2·4	—	—
			310	0·6	260	2·5	—	—
					310	0·4	—	—
Medazepam	4·4		255	3·2	233	2·9	—	—
			290(s)	1·4	252(s)	2·5	—	—
Oxazepam	1·7	11·6	239	3·6	231	4·2	236	2·9
			288	1·6	255(s)	1·8	260(s)	1·9
					280	0·8		
					310(s)	0·4		
Nitrazepam	3·2	10·8	217(s)	2·0	217	2·6	228	2·6
			282	2·6	260	1·7	260(s)	1·5
					313(s)	1·2	370	1·4
Lorazepam	1·3	11·5	240	0·8	215(s)	2·8	232	2·8
			292	3·2	231	3·6	278(s)	0·8
					259(s)	1·2		

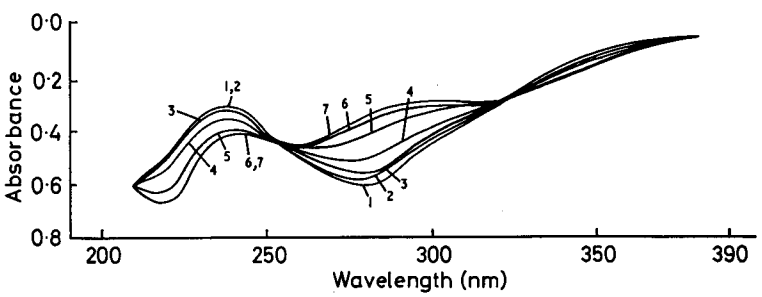


FIG. 1. pH-Dependence of ultraviolet spectra of $2{\cdot}6 \times 10^{-5}$ M solutions of nitrazepam in B.R. buffers: (1) 2·0, (2) 2·2, (3) 2·45, (4) 2·75, (5) 3·40, (6) 4·20, (7) 5·0.

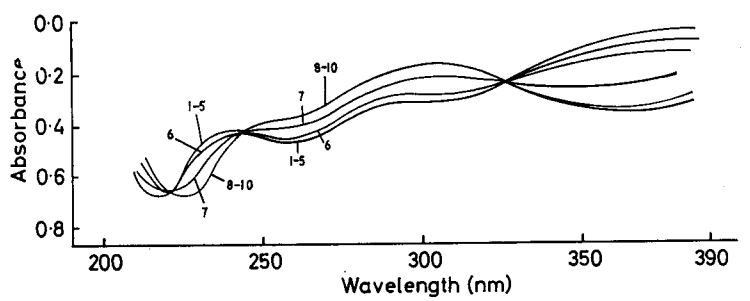


FIG. 2. pH-Dependence of ultraviolet spectra of $2{\cdot}6 \times 10^{-5}$ M solutions of nitrazepam in buffers: (1) 6·4, (2) 6·6, (3) 8·1, (4) 8·9, (5) 9·7, (6) 10·05, (7) 10·78, (8) 11·6, (9) 11·9, (10) 12·05.

the type of spectral changes occurring with varying pH for $2{\cdot}6 \times 10^{-5}$M solutions of nitrazepam in acidic and alkaline media, and illustrate the existence of pK_1 and pK_2 for this compound.

The plots of absorbance *vs* pH are shown in Fig. 3 for each of the compounds studied, the points of inflexion providing the pKa values.

Useful diagrams of $\log_{10}$ (concentration) *vs* pH for each species can be constructed from pKa data. The appropriate diagram for nitrazepam is shown in Fig. 4. Concentrations of species at any pH can be directly read from such a diagram and can be particularly useful in solvent extractions.

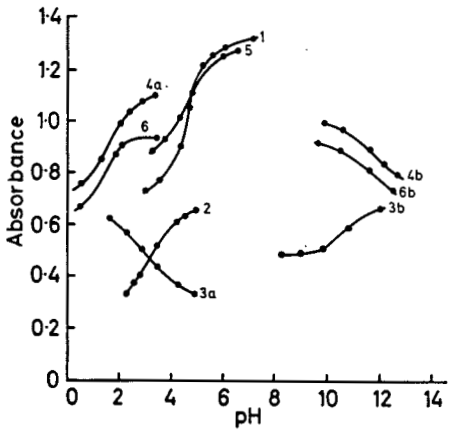


FIG. 3. Absorbance *vs* pH for benzodiazepines (1) medazepam, 5×10^{-5}M, 225 nm; (2) diazepam $2{\cdot}5 \times 10^{-5}$ M, 220 nm; (3) nitrazepam, $2{\cdot}6 \times 10^{-5}$ M, (a) 280 nm; (b) 230 nm; (4) oxazepam, (a) $2{\cdot}5 \times 10^{-5}$ M, 230 nm, (b) $2{\cdot}8 \times 10^{-5}$ M, 230 nm; (5) chlordiazepoxide, 5×10^{-5} M, 265 nm; (6) lorazepam, $2{\cdot}5 \times 10^{-5}$ M (a) 230 nm, (b) 232 nm.

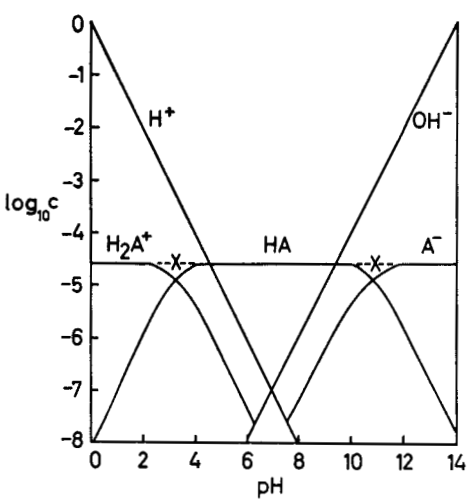


FIG. 4. Log_{10} c *vs* pH for nitrazepam.

DISCUSSION

Spectra

In general terms the spectra of the six 1,4-benzodiazepines may be understood by considering them to be the superimposed spectra of two substituted benzene rings within the complete molecule. These two are the singly substituted benzene ring on position 5 of the diazepine ring system and the triply substituted benzene ring adjoining positions 1 and 5.

To a first approximation the spectra of mono-substituted benzenes may be understood in terms of a shift to longer wavelengths of the 203·5 and 254 nm bands of benzene (Jaffe & Orchin, 1962). The spectra of polysubstituted benzenes result from an addition of the various shifts caused by the individual substituents. With such evidence in mind it is possible to calculate the shifts which may be associated with the various substituent groups in the 1,4-benzodiazepines and which are necessary to explain the appearance of two major intense bands. The low wavelength bands are produced by the shifting of the 203·5 nm band of benzene to longer wavelengths by the effect of >C = N– or >C = N→O substitution on the benzene ring in position 5

The longer wavelength bands are produced by the shifting of the 203·5 nm band of benzene by the triple substitution of the other benzene ring by the former azomethine group, by a chlorine atom or by a nitro-group (nitrazepam) in position 7. The nitrogen atom in position 1 may be an amine or amido-type.

The shifts produced by these various substituent groups in the six 1,4-benzodiazepines are shown in Table 2. Also in Table 2 are the shifts produced by these groups,

Table 2. *Shifts in the 203·5 nm band of benzene.*

Group	Shift (nm)	Observed literature shift (nm)*
Cl–	6	6
NO_2–	63·3	65
>C = N–	28	—
CH_3 O \ // N–C / \ –N = C<	18·2	35·5
	56	—
>C = N→O	46·5	—
CH_3–N<	14·5	26·5

* Aqueous systems with traces of methanol where necessary for solubility.

where known, as reported in the literature. For the chlorine atom and the nitro-group, the literature values and those calculated in the present work are in excellent agreement. The shifts produced by the amido- and amino-groups are somewhat less than previously reported. This is believed to be due to the particular geometry of the diazepine ring. It is such that full participation of the nitrogen orbitals, in causing delocalization of electrons between the group and the benzene ring, is not possible.

For nitrazepam and lorazepam there are very intense bands at 217 nm and 215 nm respectively and these are interpreted as the shifted 180 nm band of trisubstituted benzene.

Although shifts of a similar nature will occur to the 254 nm bands of the two benzene rings it is not possible to analyse the spectra successfully for these shifts since the bands themselves are of much lower intensity and are overlain by the more intense bands already dealt with. In addition any $n \rightarrow \pi^*$ or $n \rightarrow \sigma^*$ types of transitions due to the heteroatoms are all of low intensity and will not contribute significantly to the observed spectra.

The final point which needs to be made in the understanding of the spectra of the neutral 1,4-benzodiazepine molecule is that there is no significant interaction between the two benzene rings. It is obvious from models of the molecules that the two rings are by no means coplanar and only then would there be any serious inter-ring effects.

Sites of protonation and deprotonation

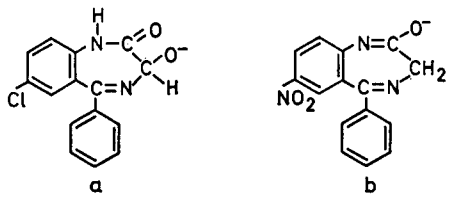
If the spectral interpretation is valid, it is possible to predict the spectral changes resulting from protonation at various sites, principally at nitrogen atoms in positions 1 and 4. For diazepam, protonation at position 1 should cause a blue shift of 18 nm of the 253 nm band corresponding to the removal of the participation of the amido-group in delocalization. In fact, there is a general shift to longer wavelengths which is possibly due to protonation at position 4, causing sufficient changes in geometry to make the delocalization of the $>C = N-$ group with the two benzene rings more effective.

Protonation at position 1 for medazepam should cause a blue shift of 14·5 nm of the 252 nm band. Again position 4 is most probable as the site of protonation since both bands undergo a red shift. A similar case exists for nitrazepam insomuch as the 260 nm band is moved to 282 nm.

The oxazepam and lorazepam molecules have an additional possible site of protonation on the 3-OH group. In both cases the two major bands are shifted towards the red end of the spectrum. Protonation at position 1 would be expected to cause a blue shift of the 255 and 259 nm bands respectively whereas protonation on the hydroxyl group in position 3 would not be expected to cause any spectral changes. Therefore position 4 is indicated as the probable site of protonation for oxazepam and lorazepam and as already discussed for diazepam, medazepam and nitrazepam.

The spectrum of chlordiazepoxide shows a blue shift of only the 260 nm band to 245 nm. This is consistent with protonation of the *N*-oxide oxygen atom, where, from Table 2, a blue shift of (46·5–28) nm is expected.

Deprotonation, in pH range 7–12 is only observed for oxazepam, lorazepam and nitrazepam. Oxazepam and lorazepam show a slight red shift at pH 11–12. Deprotonation at postion 1 or of the hydrogen atom of C–OH in position 2 (formed by enolization) or of C–H in position 3 would result in significantly larger shifts than are observed. It is concluded that the hydrogen atom is lost in moderately strong alkali from the hydroxyl group in position 3, as in a. The band due to triple substitution, for nitrazepam (313 nm), undergoes considerable red shift at pH 10–11 to 370 nm. This is consistent with the formation of a $-N = C\langle^{O^-}$ group which by delocalization would be expected to cause a considerable red shift. This is the only anion of the group that shows visible absorption. The resulting anion can be represented as in b.



Explanation of observed pKa values

Oxazepam and lorazepam have rather low values for pK_1 (1·7 and 1·3 respectively) compared with nitrazepam and diazepam (3·2 and 3·3 respectively). Since protonation occurs at position 4, and since the former two compounds have an α–OH group, the difference in pKa values is not unexpected. The extra chlorine atom in the benzene ring in position 5 (lorazepam) has a further tendency to make the protonated form less readily produced. In agreement with the fact that other similar *N*-oxides have pKa's of 4–5, it is not surprising that chlordiazepoxide has a pKa of 4·6.

The pK_2 values of oxazepam and lorazepam (11·6 and 11·5 respectively) are significantly higher than that of nitrazepam (10·8). This is expected since the charge on the anion of nitrazepam (b) is extensively delocalized by comparison with that on the anions of oxazepam (a) and lorazepam.

Acknowledgements

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