Spectroscopic Studies on Ranitidine—its Structure and the Influence of Temperature and pH

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Spectroscopic studies on ranitidine (**1**) show that the nitroethene dialkylamine group in both the base and hydrochloride exists virtually exclusively in the enediamine tautomeric form and a low barrier to rotation about the carbon–carbon double bond can be demonstrated. The energy barrier to this rotation is partly influenced by intramolecular hydrogen bonding between the nitro and amino groups. In strongly acid solution, *C*-protonation with charge delocalisation occurs on the enediamine with consequent loss of conjugation. Two polymorphic forms of the hydrochloride have been identified by i.r. spectroscopy.

Ranitidine, (*N*-{2-[({5-[(dimethylamino)methyl]-2-furanyl}-methyl)thio]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine) (**1**) is a selective H_2-receptor antagonist and a powerful inhibitor of gastric acid secretion recently introduced for the treatment of peptic ulcers and related disorders.[1–4] A key feature of the structure is the 1,1-bisalkylamino-2-nitroethene moiety. Recent publications[5,6] purport to show that this fragment of ranitidine exists in the nitronic acid form (**2**) and suggest that this may have a bearing on the interaction of ranitidine with the cytochrome P-450 mixed function oxygenase enzyme system in the liver (an interaction shown to be weak in the case of rat microsomal cytochrome P-450 *in vitro*[7] and concluded to have negligible effect on the metabolism of other drugs in the clinical situation[8]). Our own work, which is described in this paper, shows that the nitronic acid form is not a correct portrayal and that the true structure of ranitidine is represented by (**1**). This also accords with the findings in these laboratories that all attempts to achieve *O*-alkylation, which in (**2**) would be expected to occur, have been unsuccessful.

Nitroenamines (1-amino-2-nitroethenes), including nitroketene aminals (1,1-diamino-2-nitroethenes), are a well known class of compounds whose synthesis, structure, and reactivity have been recently reviewed.[9] In theory, ranitidine could exist in one of the three main tautomeric forms (Figure 1). In addition, the enamine could exist as two geometrical isomers and the nitronic acid and imine forms could both exhibit further prototropic tautomerism within the amidine group as well as *syn–anti* isomerism about the C=N bonds. The imine form (**3**) can be immediately discounted on the basis of the n.m.r. spectrum which shows a one-proton singlet in the olefinic region but no signal due to a nitromethylene group.

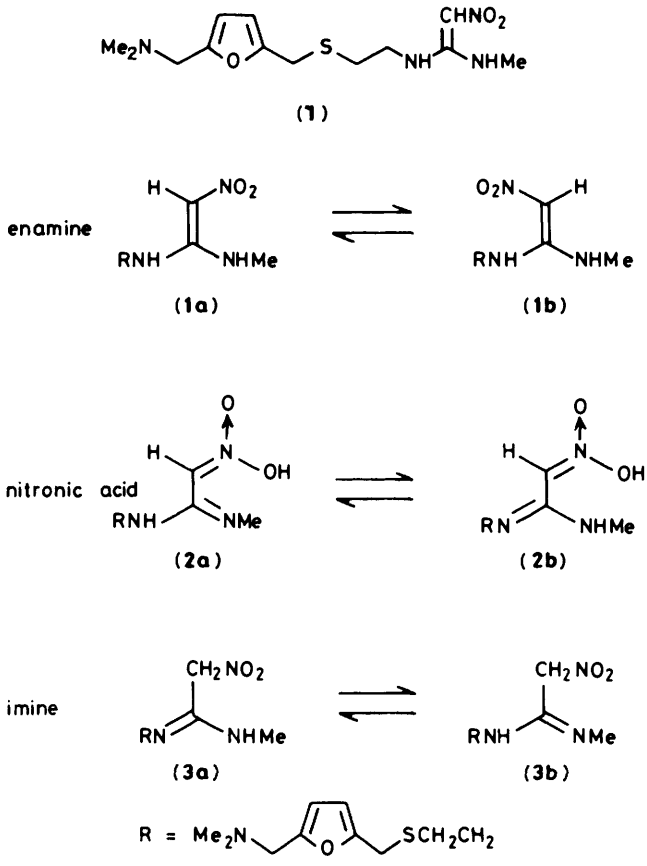


Figure 1. Possible tautomers of ranitidine

Experimental

Materials.—Ranitidine free base (m.p. 72 °C†) and hydrochloride (polymorph 1, m.p. 144.5 °C, and polymorph 2, m.p. 146.2 °C, as measured by differential scanning calorimetry) were synthesised by the Chemical Development, Glaxo Group Research Ltd., Ware, and 1,1-bis(methylamino)-2-nitroethene (**4**) by the Chemical Research Department.

Measurements.—U.v. spectra were obtained on a Perkin-Elmer 402 or a Hewlett-Packard HP8450A spectrophotometer. I.r. spectra were obtained on a Perkin-Elmer 357 or 377 spectrophotometer. ^{1}H N.m.r. spectra were obtained on a Varian EM390 (90 MHz) or Bruker WM250 (250.13 MHz) spectrometer and ^{13}C n.m.r. spectra on the latter (62.90 MHz) or a JEOL FX100 instrument (25.1 MHz). Accurate m.p.s of the polymorphs of ranitidine hydrochloride were obtained on a Perkin-Elmer DSC-2C differential scanning calorimeter.

Results and Discussion

U.v. Spectra and Ionisation Behaviour.—Ranitidine is a base with a pK_a (measured by titration) of 8.2 and the ^{1}H n.m.r. spectra of the base and hydrochloride show quite clearly that the basic centre is the dimethylamino group. In water at pH 6.5 in which the base is virtually completely ionised, the u.v. spectrum (Figure 2) has $\lambda_{max.}$ 229 (ε 16 300) and 315 nm (ε 15 400). The lower wavelength maximum is due mainly to the

† The m.p. of ranitidine free base is erroneously recorded as 95.5—96.0 °C in 'Annual Drug Data Report,' ed. J. R. Prous, Prous, Barcelona, 1982, vol. 4, p. 168.

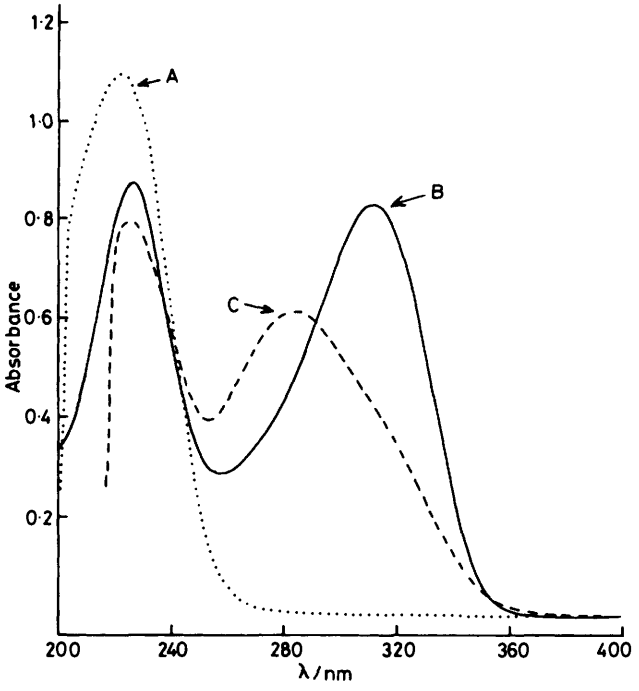


Figure 2. U.v. spectra of ranitidine as a function of pH: A, M-HCl; B, H_2O; C, M-NaOH

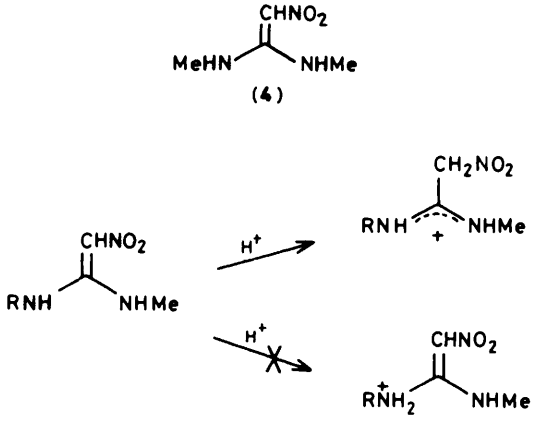


Figure 3. Protonation of the diaminonitroethene group of ranitidine

disubstituted furan chromaphore with a contribution from the diaminonitroethene group which has its main absorption at 315 nm; *cf.* 1,1-bis(methylamino)-2-nitroethene (**4**); $\lambda_{max.}$ 226 (ε 4 200) and 313 nm (ε 15 200). Although the u.v. spectrum is unaffected by protonation of the dimethylamino group, in stronger (*e.g.* M-hydrochloric) acid the band at 315 nm disappears completely, indicating that protonation of the dialkylaminonitroethene group leads to loss of conjugation (Figure 3). The pK_a for this process, determined spectrophotometrically, is 2.3. This result suggests that *C*-protonation is taking place rather than *N*-protonation which would lead to a reduction rather than complete loss of conjugation.

A further change in the u.v. spectrum occurs when ranitidine is dissolved in alkali. The maximum shifts from 315 to 285 nm as a result of the dissociation of a weak acid. This is only complete in 5M-sodium hydroxide and its pK_a has not been accurately measured but it is in the region of 14. Presumably proton loss is from nitrogen although why it leads to a hypsochromic shift is not clear.

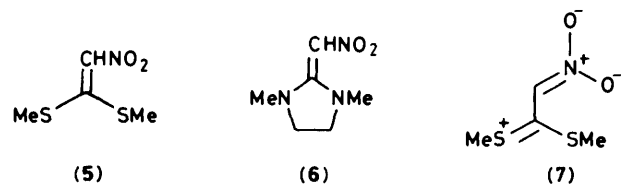


Table 1. ^{1}H N.m.r. data[a] at slow exchange and free energy barriers to rotation

Compound	Signal studied	Solvent	T/K	T_c/K	Δν/Hz	$\Delta G^{\ddagger}$/ kcal mol^{-1}
(**1**) as base	NH*Me*	$CDCl_3$	271	314	32.5	15.7 ± 0.1
(**1**) as base	NH*Me*	[$^2H_4$]MeOH	213	258	25.5	13.0 ± 0.2
(**1**) as HCl	NH*Me*	[$^2H_4$]MeOH	240	262	30.5	13.1 ± 0.2
(**4**)	NH*Me*	$CDCl_3$	270	306	18.0	15.7 ± 0.2

[a] 250 MHz spectrometer.

^{1}H *N.m.r. Spectra.*—The ^{1}H n.m.r. spectra of both ranitidine and its hydrochloride salt show temperature-dependent variations consistent with a low barrier to rotation about the C(1)–C(2) bond of the dialkylaminonitroethene group. This type of n.m.r. behaviour has been observed[10] in ketene mercaptals and aminals such as (**5**) and (**6**) where the low barrier to rotation is ascribed to the lowered bond order of the double bond due to the contribution from polar limiting structures such as (**7**). The *X*-ray crystal structure of ranitidine base[11] shows that there is similar extensive delocalisation within the dialkylaminonitroethene group since the bond lengths of the C(1)–C(2) bond, the two C–NHR bonds, and the C–NO_2 bond are intermediate between single and double.

In view of their differing solubilities, the n.m.r. spectrum of the base was run in deuteriochloroform and that of the hydrochloride in deuterium oxide or deuteriomethanol and it is convenient to deal with them separately.

Ranitidine base. At 90 MHz, the spectrum at 328 K (Figure 4) in $CDCl_3$ shows one set of time-averaged signals due to fast interconversion of the rotamers [(**1a**)⇌(**1b**)]. At 250 MHz and 328 K, the signals for the NHCH_2 and NHCH_3 do not collapse completely (Figure 5). This is a consequence of higher coalescence temperatures associated with higher field strengths. At 271 K the interconversion is much slower and two sets of signals are observed for all the protons associated with the dialkylaminonitroethene group. The measured coalescence temperature, T_c, at 250.13 MHz for the NHCH_3 signals, is 314 K and the energy barrier to rotation as given by the difference in Gibbs free energy, $\Delta G^{\ddagger}$, is 15.7 ± 0.1 kcal mol^{-1} calculated using equations (1)—(3).[12]

$$\Delta G^{\ddagger} = 4.575 \times 10^{-3} T [10.319 + \log (T/k)] \quad (1)$$

$$\text{At coalescence, } k_c = \pi \delta\nu/\sqrt{2} \quad (2)$$

δν is the frequency difference in Hz between the two sites in the system in the absence of exchange, *i.e.* below coalescence. Combining (1) and (2) gives (3). This approximation method is

$$\Delta G^{\ddagger} = 4.575 \times 10^{-3} T_c [9.972 + \log (T_c/k_c)] \quad (3)$$

only applicable where there are two sites with almost equal populations and this appears to be the case in this instance.

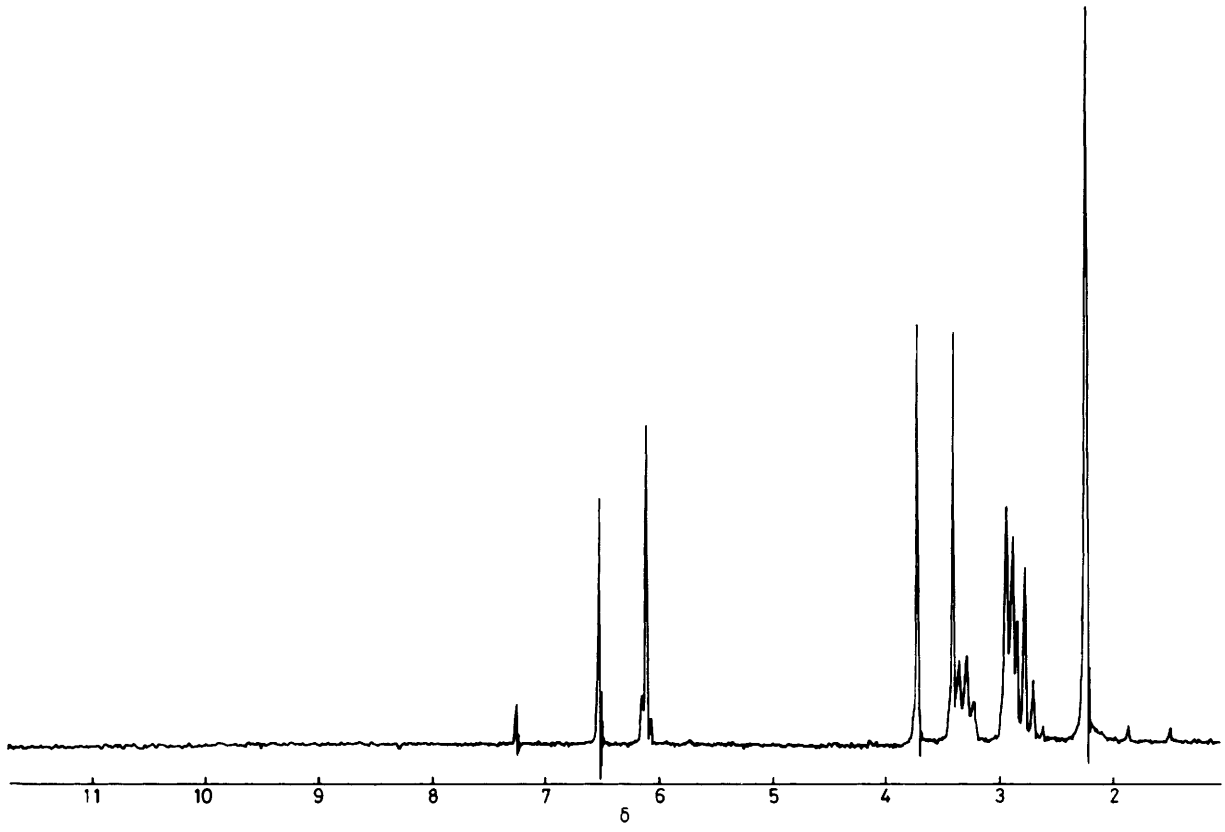


Figure 4. 90 MHz ^{1}H N.m.r. spectrum of ranitidine base at 328 K in $CDCl_3$

In $CDCl_3$, 1,1-bis(methylamino)-2-nitroethene (**4**), as expected, has a similar energy barrier ($\Delta G^{\ddagger}$ 15.7 ± 0.2 kcal mol^{-1}) to that of ranitidine.

At 250 MHz and 271 K, the spectrum of ranitidine (Figure 5) shows that the signals associated with the $NHCH_3$ and $NHCH_2$ groups display couplings. Spin decoupling experiments on the two NH quartets and two NH triplets have been carried out to confirm the assignments of the signals to the individual rotamers (Table 2).

Now it is possible to write the following equilibria between enamine and nitronic acid species (Scheme 1). The conversion of enamine into nitronic acid requires a 1,5-proton shift. The equilibria (**1a**)⇌(**1b**) and (**2a**)⇌(**2b**), although both involve rotation about the C(1)–C(2) bond, are not the same since (**2a**)⇌(**2b**) requires a 1,3-proton shift in the 'amidine' group as well. Since N-H coupling is still observed to both CH_3 and CH_2 under conditions of rapid rotation about C(1)–C(2), (**1a**) and (**1b**) must be involved as (**2a**) and (**2b**) are not directly interconvertible. This situation may be compared to that of β-ketoaldehydes where interconversion between the two enol forms (Scheme 2) is rapid, leading to a time-averaged n.m.r. spectrum.

In an investigation of a series of cyclic β-ketoaldehydes,[13] it was found that the magnitude of the coupling constant, $3J_{H,COH_b}$, is a linear function of the proportion of hydroxymethylene–ketone form. Thus, in the case of ranitidine, the fact that $3J_{HNCH_3}$ and $3J_{HNCH_2}$ (*ca.* 7 Hz) are not at all attenuated from normal values suggests that the nitronic acid tautomers (**2**) do not exist to any significant extent.

Ranitidine hydrochloride. At 90 MHz and in D_2O, only one set of signals above 5 °C is observed with no line-broadening. This suggests that the energy barrier to rotation about the carbon–carbon double bond is much lower in D_2O than in $CDCl_3$. This is not unexpected since intramolecular hydrogen-bonding which presumably stabilises the individual rotamers (**1a**) and (**1b**) in non-polar solvents is likely to be disrupted in a polar solvent such as D_2O. Although the signals due to separate rotamers could not be observed in D_2O, in [$^2H_4$]MeOH at 240 K separate N-Me signals are observed. The measured coalescence temperature at 250.13 MHz. for the N-CH_3 signal is 262 K and the energy barrier, $\Delta G^{\ddagger}$, is 13.1 ± 0.2 kcal mol^{-1}. This is comparable to that found for ranitidine base in [$^2H_4$]MeOH ($\Delta G^{\ddagger}$ is 13.0 ± 0.2 kcal mol^{-1}). The reduction in energy barrier between the base and hydrochloride in [$^2H_4$]MeOH, and base in $CDCl_3$, of *ca.* 3 kcal mol^{-1} is probably a measure of the intramolecular hydrogen bond strength.

Ranitidine 'dihydrochloride'. Although the dihydrochloride of ranitidine has not been isolated, in the presence of excess of hydrochloric acid, protonation of the dialkylaminonitroethene moiety (pK_a 2.3) also takes place and the effect of this can be

Table 2. ^{1}H N.m.r. data of ranitidine base[a]

	328 K Time averaged	271 K Rotamer (**1a**)	271 K Rotamer (**1b**)
CH_2NH	3.34br	3.24	3.42
CH_2NH	v. br	6.49	10.30
CH_3NH	v. br	10.23	6.90
$C{=}CHNO_2$	2.92br	3.00	2.88
	6.56	6.60—6.62	

Chemical shifts in p.p.m (δ) from Me_4Si.

[a] 4% w/v solution in $CDCl_3$ run at 250 MHz.

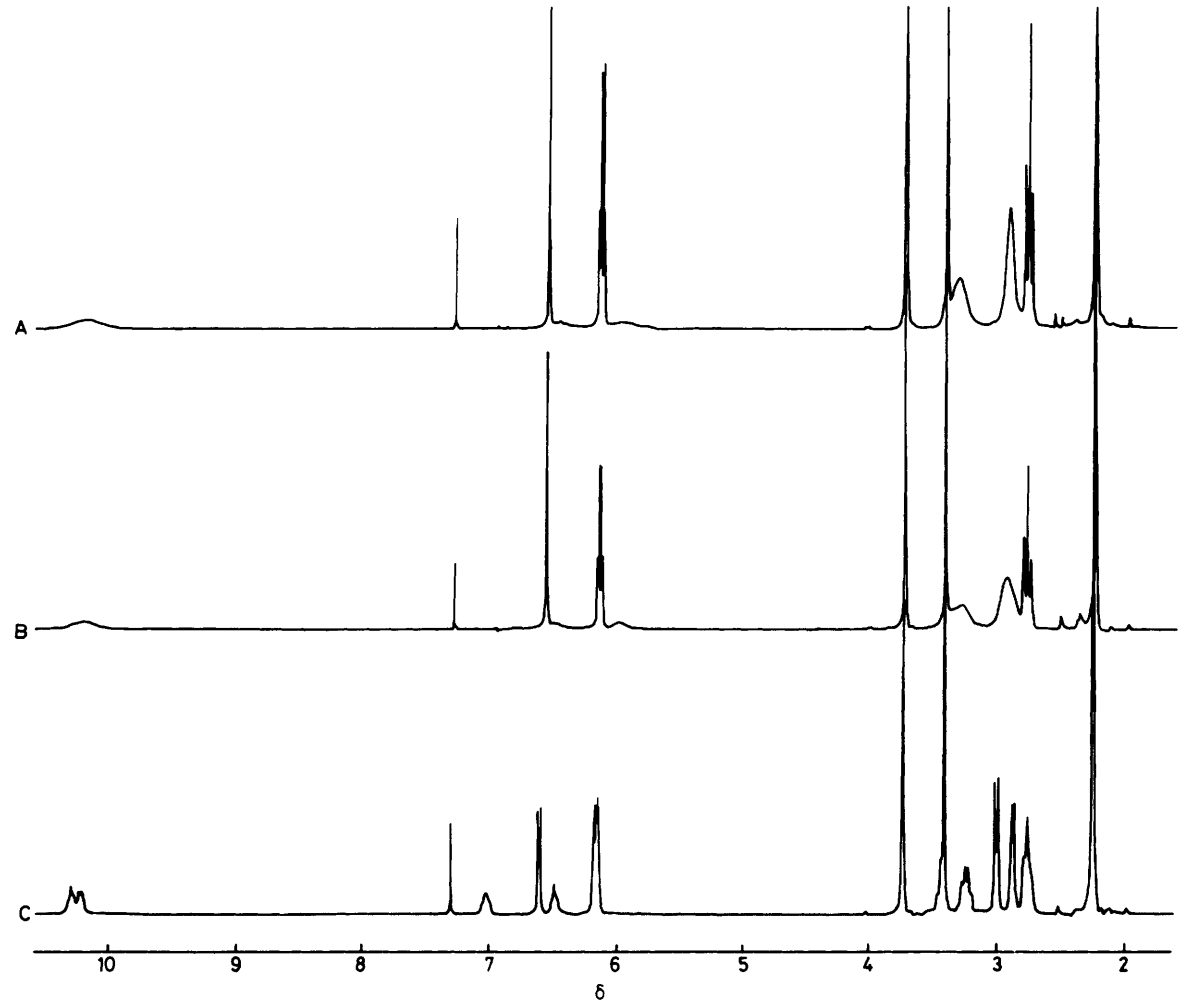
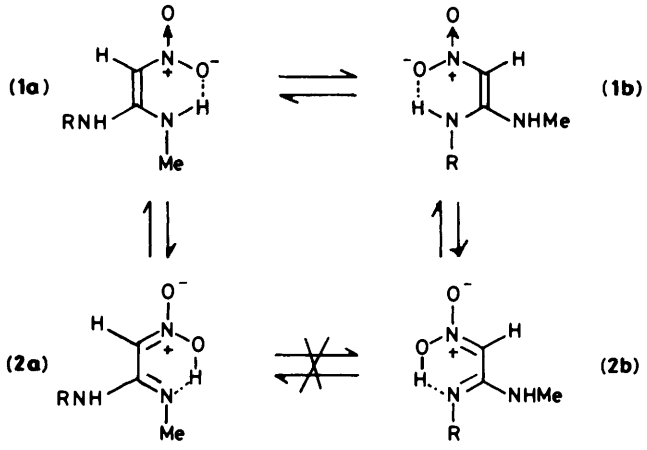
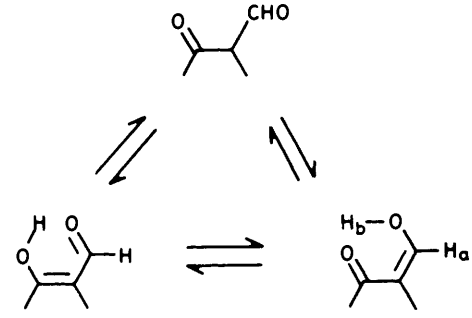


Figure 5. 250 MHz ^{1}H N.m.r. spectra of ranitidine base in $CDCl_3$ at various temperatures: A, 328 K; B, 314 K; C, 271 K



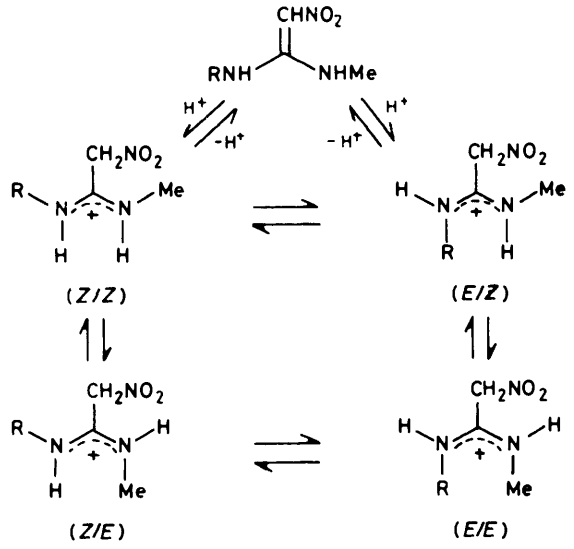
Scheme 1.



Scheme 2.

seen in the n.m.r. spectrum. As HCl is progressively added to an aqueous solution of ranitidine hydrochloride, the olefinic signal at δ 6.83 is gradually replaced by a nitromethylene signal at δ 5.80, thus confirming that *C*-protonation rather than *N*- or *O*-protonation takes place (Figure 3). At the same time, the N-CH_3 signal at δ 2.94 progressively disappears, to be replaced by *two* N-CH_3 doublets at δ 3.13 and 3.03 and the N-CH_2 quartet and S-CH_2 triplet are similarly replaced by double signals in diprotonated ranitidine. Two points arise here: first, signals due to both mono- and di-protonated ranitidine can be seen simultaneously, therefore the second protonation–deprotonation step must be a slow (on the n.m.r. time-scale) exchange process in contrast to *N*-protonation, and second, the doubling

of signals in the diprotonated species must be explained. It cannot be due to restricted rotation about the C(1)–C(2) nitroethene bond as in ranitidine base because the conjugation between nitro and amino groups, which strongly stabilised the planar rotamers, is no longer present. Although intramolecular hydrogen bonding might influence the energy barrier to rotation, such bonding is far too weak to account for the high energy barrier seen here (no coalescence of signals was observed up to 95 °C in 2M-D_2O–DCl). The explanation lies in the structure of the protonated nitroethene group (Scheme 3) which is an amidinium salt. In such salts, delocalisation of the positive charge results in two C–N bonds of equal order [14] hence there are four theoretically possible geometrical isomers: *Z*/*Z*, *E*/*Z*, *Z*/*E*, and *E*/*E* which, if they were all present, would give rise to four $NHCH_3$ and four $NHCH_2$ signals in the n.m.r. spectrum. In practice only two of each are observed and in an n.O.e. difference experiment carried out in H_2O–HCl, saturation of the nitromethylene group gave an n.O.e. for only *one* of the $NHCH_3$ and *one* of the $NHCH_2$ signals. It is therefore reasonably certain that diprotonated ranitidine exists as an approximately equimolar mixture of *E*/*Z* and *Z*/*E* isomers (which are likely to be of very similar energy). The alternative explanation, that it is an equimolar mixture of *Z*/*Z* and *E*/*E* isomers, is highly unlikely in view of the much greater steric hindrance involved and perhaps also the lack of hydrogen bonding possibilities for the *Z*/*Z* isomer. The same conclusion was reached by Neuman *et al.*[15] in an n.m.r. investigation of *NN*-dimethylacetamidinium chloride and our own study of the behaviour of the symmetrical dimethylaminonitroethene (**4**) in aqueous acid also supports this view.



Scheme 3.

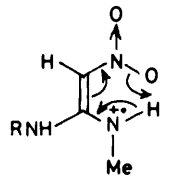


Figure 6. Proposed mechanism for the loss of OH from ranitidine molecular ion

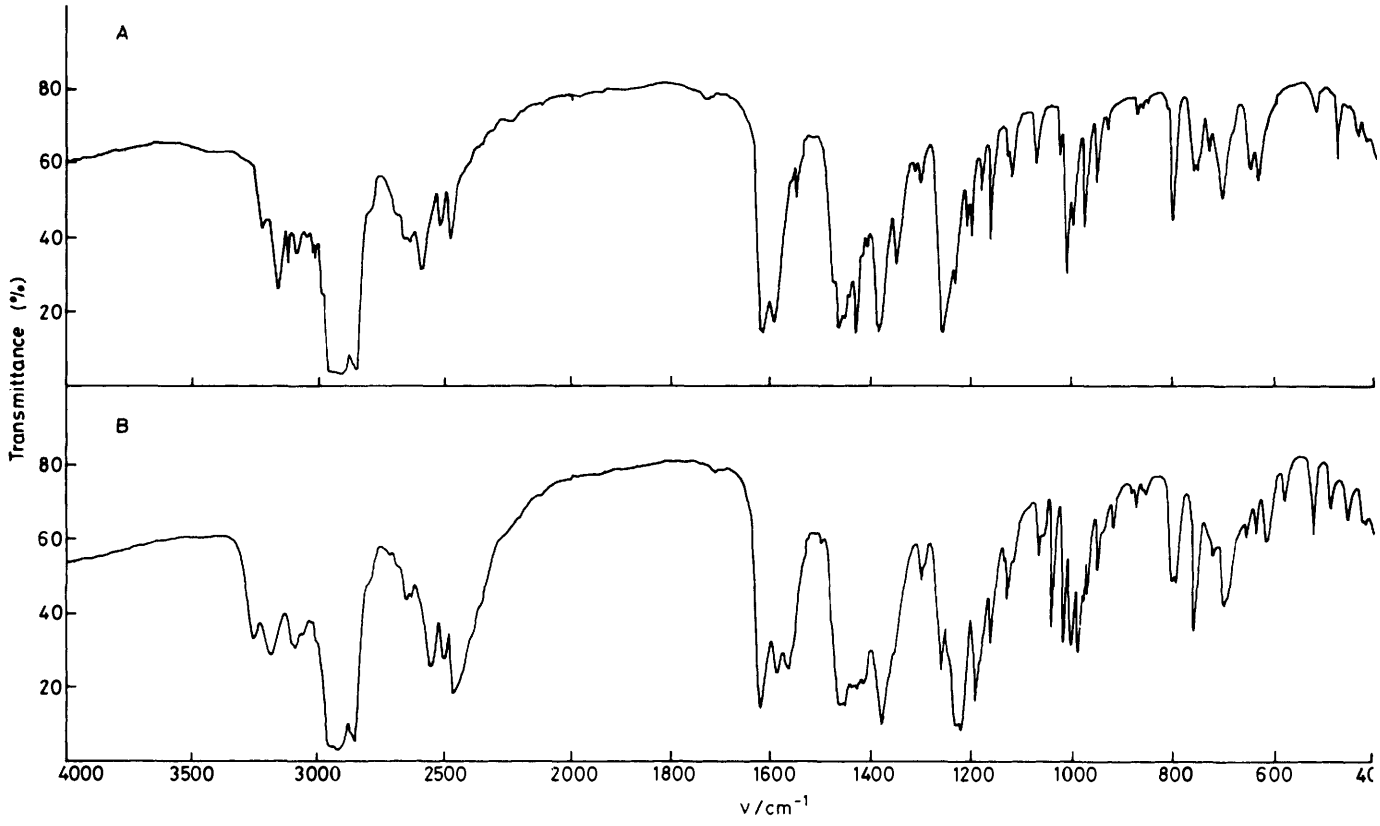
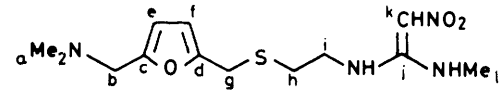


Figure 7. I.r. spectra of the two polymorphs of ranitidine. A, m.p. 144.5; B, m.p. 146.2 °C

Table 3. ^{13}C N.m.r. data of ranitidine



Assignment	*a*	*b*	*c*
a	44.7	44.6	45.2
b	55.7	55.3	55.9
c	151.7, 150.3	156.4	156.6
d		145.6	146.0
e	109.1, 108.1	118.0	118.5
f		111.7	112.3
g	28.1	30.1	30.5, 30.8
h	30.5	33.0	31.6, 33.6
i	40.3	43.2	47.2, 45.2
j	156.2	158.3	158.0
k	97.9	102.6	74.6*
l	28.1	30.8	33.6, 32.6

[a] Base in $CDCl_3$, chemical shifts (p.p.m.) downfield from Me_4Si. [b] Hydrochloride in D_2O, chemical shifts (p.p.m.) downfield from sodium trimethylsilylpropanesulphonate. [c] Hydrochloride in 2M-HCl, chemical shifts (p.p.m.) downfield from sodium trimethylsilylpropanesulphonate.

* CH_2NO_2.

Ranitidine anion. The n.m.r. spectrum of ranitidine in M-NaOD shows upfield shifts of 0.10 and 0.20 p.p.m. for the $NHCH_3$ and $NHCH_2$ signals respectively compared with ranitidine in D_2O. These shifts are consistent with increased electron density at the amine nitrogen atoms due to anion formation. As one might predict, exchange of the olefinic proton in D_2O–NaOD is very much slower than at lower pH.

^{13}C *N.m.r. Spectra.*—The ^{13}C n.m.r. data for ranitidine base, hydrochloride and 'dihydrochloride' are given in Table 3. The assignments are straightforward and consistent with the enamine structure[16] which is inferred from the ^1H n.m.r. spectra. The carbon signals associated with the dialkylaminonitroethene moiety show marked broadening at 20 °C, again evidence for slow rotation about the C(1)–C(2) double bond. In addition, the spectrum of the hydrochloride in aqueous hydrochloric acid shows a methylene carbon (multiplicity obtained from the single-frequency off-resonance decoupled spectrum) at δ 74.6 p.p.m. and no olefinic methine carbon in the 100 p.p.m. region, consistent with protonation at C(2).

Mass Spectra.—The mass spectrum of ranitidine hydrochloride, obtained under conditions of electron impact (e.i.) ionisation, did not contain a molecular ion. However, under the milder conditions of chemical ionisation using ammonia as the reagent gas, an $(M + H)^+$ ion was observed at *m/z* 315.1495 (calc. for $C_{13}H_{23}N_4O_3S$: *M*, 315.1491). The ready loss of OH from the molecular ion in the e.i. spectrum is not as claimed[5] evidence specifically for the nitronic acid form since the enamine form could equally well fragment with loss of OH (Figure 6). Indeed this is the most common mode of fragmentation in many nitro compounds.[17]

The I.r. Spectra of Ranitidine.—The base in the solid state (mull in mineral oil) and in strong (10%) solutions in chloroform shows bonded NH absorption ($\nu_{max.}$ 3 270 and 3 200 cm^{-1}).

Free NH absorption at 3 450 cm^{-1} in solution increases with increasing dilution but bonded forms are still present, a peak at 3 260 cm^{-1} being the most prominent at highest dilutions (0.1% in $CHCl_3$ or $CDCl_3$ and 0.005% in CCl_4).

The main peaks due to a 2,5-disubstituted furan can be identified at 1 015 and 790 cm^{-1} and the dimethylamino group gives typical Bohlmann bands at 2 820 and 2 780 cm^{-1}.

The spectra of ten simple 1,1-bisalkylamino-2-nitroethenes have been examined[18] including 1,1-bis(dimethylamino)-2-nitroethene which cannot exist in the tautomeric nitronic acid form. Characteristic peaks common to all these can be recognised in ranitidine base at 1 610, 1 565, 1 380, 1 250, and 760 cm^{-1}, and most significantly the small sharp ethylenic CH stretching peak at 3 160 cm^{-1}, visible in solution and in the solid state, confirms the presence of the nitroethene structure. Doubt attaches to the assignment of *as*- and *sym*-NO_2 stretching frequencies in nitroalkene systems[19] and they can provide no convincing evidence to favour any of the possible tautomeric structures in ranitidine.

From i.r. spectra (Figure 7) obtained from the solid state (mulls in mineral oil and KCl discs), two polymorphic forms of ranitidine hydrochloride (m.p.s 144.5 and 146.2 °C) have been characterised. The spectra differ considerably in detail especially in the region above 3 000 cm^{-1} (bonded NH absorption), the complex peaks of the protonated dimethylamino group in the 2 700—2 300 cm^{-1} region, and in the 1 620—1 570 cm^{-1} region.

Acknowledgements

We thank Dr. R. J. Tanner for mass spectral data and Dr. R. Fletton, Glaxo Group Research (Greenford), for assistance on preliminary ^{13}C n.m.r. work.

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Received 19*th January* 1984; *Paper* 4/100