Proton Transfer from Heterocyclic Compounds. Part I. Some Benzimidazoles

By **John A. Elvidge, John R. Jones,*** and **Conor O'Brien,** Chemistry Department, University of Surrey, Guildford
E. Anthony Evans and **John C. Turner,** The Radiochemical Centre, Amersham

Measurements are reported at 85° on the rates of detritiation of several benzimidazoles specifically labelled at C-2. Rate–pH profiles as well as the effects of substituents on rates suggest that the mechanism is the same as that operating in other five-membered heterocyclic compounds, namely an initial protonation at the basic N-3 followed by a rate determining abstraction of tritium by hydroxide ions to give an ylide intermediate. The results for [2-^{3}H]-1,3-dimethylbenzimidazolium bromide support this conclusion.

STUDIES of the rates of ionisation of carbon acids such as ketones and nitro-compounds have contributed greatly to our understanding of proton transfer reactions in solution. Considerably less work has however been done on heterocyclic compounds although such work could be of benefit in three areas. First, it may be possible to separate the contributions from inductive, coulombic, resonance, *d* orbital, and *s* character effects to the rates. Secondly, it may provide information of synthetic use, and finally, as many of the compounds serve as substrates in enzymatic processes it could lead to a better understanding of the specificity of enzymes.

In the present work isotopic hydrogen exchange using tritium as a tracer has been employed as a means of measuring the rates of ionisation. Because of the difficulties arising from limited solubility, and slow exchange rates, we have preferred a higher temperature (85°) than is customary in such work. The ease and accuracy with which the data can be obtained more than compensates for the disadvantages of operating at such a temperature.

We have previously published our preliminary findings for purine, adenine, adenosine, and benzimidazole.[1] In the present paper the full results for benzimidazole as well as some substituted benzimidazoles are presented. Several studies relevant to this work and to the general problem of isotopic exchange at C-2 in five-membered heterocyclics have been reported recently. The kinetics of ionisation in aqueous solution of the hydrogen atom at C-2 of for example, thiazole [2] and imidazole [3] both indicate a common mechanism which involves protonation at the adjacent N-3 followed by abstraction of the hydrogen at C-2 by hydroxide ions with the formation of an ylide intermediate. Similar species have also been postulated to account for the ready isotopic exchange that occurs in six-membered rings.[4]

Previous work in the benzimidazoles has been somewhat limited. Fritzsche [5] observed that on prolonged heating in D_2O exchange occurred at C-2 and recently the rates of exchange for this compound as well as 1-methylbenzimidazole have been measured [6] in EtOD.

EXPERIMENTAL

Materials.—Benzimidazole (1a) was purchased commercially. 5,6-Dichloro- [7] (1e), 4,5,6-trichloro- [8] (1f), and 4,5,6,7-tetrachlorobenzimidazole [9,10] (1g) were kindly provided by Professor G. T. Newbold. The 1-methyl- (1b), 1-ethyl- (1c), and 1-isopropyl- (1d) benzimidazoles were synthesised by a standard alkylation procedure [11] and characterised by the preparation of the corresponding picrates.

1,3-*Dimethylbenzimidazolium Bromide* (2).—1-Methylbenzimidazole (1b) was refluxed in xylene with an excess of bromomethane for 1 h. The *crystals* that were precipitated and isolated in 60% yield were recrystallised three times from ethanol–ether, m.p. 245—246°, $\lambda_{max.}$ (pH 7) 263 nm (ε 5600) (Found: C, 44·1; H, 5·45; N, 11·4. $C_9H_4BrN_2,H_2O$ requires C, 44·1; H, 5·3; N, 11·4%).

All the labelled compounds were prepared by homogeneous exchange using tritiated water (5 Ci ml^{-1}). Thus [2-^{3}H]benzimidazole was prepared by maintaining a solution of substrate (*ca.* 10 mg) in tritiated water (0·1 ml) at 85° overnight. In cases where the solubility of the compound was low some dioxan was added. The solvent was removed by freeze drying, a small amount of H_2O added to exchange labile hydrogen, and the water removed also by freeze drying. The product was finally dried in a vacuum desiccator.

Kinetics.—The labelled benzimidazole was dissolved in aqueous solution (10 ml) at 85° and samples (0·5 ml) were withdrawn from the reaction vessel at intervals. The water was removed by freeze drying and its tritium content (C_t) determined by liquid scintillation counting using an NE 250 scintillator. The infinity sample (tritium content = C_∞) was taken after more than ten half-lives had elapsed. Usually the reaction was followed to greater than 80% completion and sufficient counts taken to obtain a statistical accuracy of better than ±0·2%. Periodic checks were made to ensure that no loss of tritium by evaporation occurred during any single run. Values of $\log_{10}(C_\infty - C_t)$ were plotted against time and the pseudo first-order rate

[1] J. A. Elvidge, J. R. Jones, C. O'Brien, and E. A. Evans, *Chem. Comm.*, 1971, 394.
[2] R. A. Coburn, J. M. Landesberg, D. S. Kemp, and R. A. Olofson, *Tetrahedron*, 1970, **26**, 685.
[3] J. D. Vaughan, Z. Mughrabi, and E. Chung Wu, *J. Org. Chem.*, 1970, **35**, 1141.
[4] J. A. Zoltewicz, G. M. Kauffmann, and C. L. Smith, *J. Amer. Chem. Soc.*, 1968, **90**, 5939.
[5] H. Fritzsche, *Biochim. Biophys. Acta*, 1967, **149**, 173.
[6] N. N. Zatzepina, Yu. L. Kaminskii, and I. F. Tupitsyn, *Org. Reactivity*, 1967, **4**, 177.
[7] D. J. Rabiger and M. M. Jouillie, *J. Org. Chem.*, 1964, **29**, 476.
[8] B.P. 783,306/1957.
[9] I. Tamm, K. Folkers, and C. H. Shunk, *J. Bacteriology*, 1956, **72**, 54.
[10] D. W. J. Lane and G. T. Newbold, B.P. 1,063,473/1967.
[11] A. F. Pozharskii and A. M. Simonov, *Zhur. obshchei Khim.*, 1963, **33**, 179.

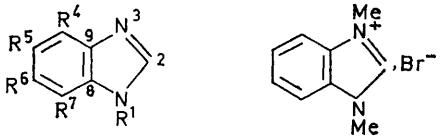
constant k_{obs} obtained from the slope. The experimental accuracy was ±3—5%.

For kinetic measurements at low and high pH, solutions of hydrochloric acid and sodium hydroxide were used respectively. For intermediate values of pH, formate and acetate buffers were used. The pH of the solutions were measured at 20° and for the formate and acetate buffers it was assumed that the values did not change on going from 25 to 85°. Several studies bear witness to the temperature independence of such buffers.[12] In other cases the variation of K_w with temperature is well known [13] and the value of pK_w at 85° is 12·50.

Equilibrium Measurements.—The pK_a (of benzimidazole for protonation of the basic N-3) and of the 1-methyl and 1-ethyl compounds were determined at 25° using a standard procedure.[14]

RESULTS AND DISCUSSION

The rates of detritiation of [2-^{3}H]benzimidazole as a function of pH exhibit a bell-shaped profile in which there is a region between the acidic and basic extremities where k_{obs} is virtually unaffected by changes in pH. Such behaviour is consistent with a mechanism in which the conjugate acid of the substrate is being attacked by the hydroxide ion in the rate-determining step. If BH, B^-, and BH_2^+ represent benzimidazole, benzimidazole anion (formed by ionisation of 1-H) and protonated



(1) (2)

a; $R^1 = R^4 = R^5 = R^6 = R^7 = H$
b; $R^4 = R^5 = R^6 = R^7 = H$, $R^1 = Me$
c; $R^4 = R^5 = R^6 = R^7 = H$, $R^1 = Et$
d; $R^4 = R^5 = R^6 = R^7 = H$, $R^1 = Pr^i$
e; $R^4 = R^7 = R^1 = H$, $R^5 = R^6 = Cl$
f; $R^7 = R^1 = H$, $R^4 = R^5 = R^6 = Cl$
g; $R^1 = H$, $R^4 = R^5 = R^6 = R^7 = Cl$

benzimidazole (formed by protonation of N-3) respectively then equation (1) holds. With $K_a = [BH][H^+]/[BH_2^+]$ and $K_a' = [B^-][H^+]/[BH]$ we find that

$$[B]_T = [BH] + [B^-] + [BH_2^+] \quad (1)$$

$$[B]_T = [BH_2^+]\left(\frac{K_a}{[H^+]} + \frac{K_aK_a'}{[H^+]^2} + 1\right) \quad (2)$$

equation (2) applies. If the rate is given by equation (3) we have equation (4). For the 1-alkylbenzimidazoles

$$\text{Rate} = k_2[BH_2^+][OH^-] \quad (3)$$

$$\text{Rate} = \frac{k_2[B]_T[H^+]}{K_a + \frac{K_aK_a'}{[H^+]} + [H^+]} \cdot [OH^-] = \frac{k_2[B]_TK_w}{K_a + \frac{K_aK_a'}{[H^+]} + [H^+]} \quad (4)$$

[12] R. P. Bell, ' The Proton in Chemistry,' Methuen, London, 1959, p. 65.
[13] H. L. Clever, *J. Chem. Educ.*, 1968, **45**, 231.

K_a' is zero so that equation (4) reduces to (5) and equation (6) is obtained. For the situation in which

$$\text{Rate} = k_2[B_T]K_w/(K_a + [H^+]) \quad (5)$$

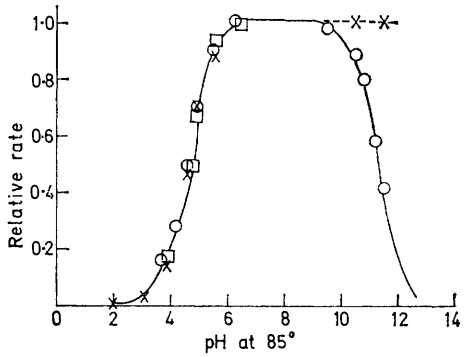
$$k_{obs} = k_2K_w/(K_a + [H^+]) \quad (6)$$

$K_a \gg [H^+]$ equation (7) is obtained which also holds for

$$k_{obs} = k_2K_w/K_a \quad (7)$$

benzimidazole itself when $K_a \gg [H^+] \gg K_a'$.

The relative rate can be defined as a fraction of k_2K_w/K_a and this is plotted against pH in the Figure.



Rate–pH profile for ○, [2-^{3}H]benzimidazole; ×, [2-^{3}H]-1-methylbenzimidazole; and □, [2-^{3}H]-1-ethylbenzimidazole. The line is drawn for benzimidazole

The calculated curve, represented by the solid line, was obtained by a trial and error procedure; k_2K_w/K_a was taken as unity and various values of K_a and K_a' inserted in equation (8) in order to bring about the best agreement between experimental and calculated values of k_{obs}. The experimentally determined pK_a values at 25° are

$$k_{obs} = \frac{k_2K_w}{K_a + \frac{K_aK_a'}{[H^+]} + [H^+]} = \frac{1}{1 + \frac{K_a'}{[H^+]} + \frac{[H^+]}{K_a}} \quad (8)$$

5·53 [15] and 13·2.[16] Typical temperature coefficients [17] for nitrogen acids of such acidities are −0·016 and −0·024 pK_a units per °C rise above 25° so that the values at 85° should be 4·57 and 11·76. In fact the calculated curve was obtained using $pK_a = 4·6$ and $pK_a' = 11·5$.

The rate–pH profile for benzimidazole differs from that obtained for imidazole [3] in that the observed rate for the latter remains virtually constant in the pH range 9—14. This difference arises from the fact that 1-H of benzimidazole is considerably more acidic than in imidazole; the generation of negative charge α to the exchanging position makes the anion unreactive to exchange. The results for 1-methylbenzimidazole, where anion formation is not possible at the higher pH values, do in fact resemble those of imidazole.

Our work did not extend to media of high acidity so that we are unable to say whether a rate-determining

[14] A. Albert and E. P. Serjeant, ' Ionization Constants of Acids and Bases,' Methuen, London, 1962.
[15] D. D. Perrin, *J. Chem. Soc.*, 1965, 5590.
[16] G. Yagil, *Tetrahedron*, 1967, **23**, 2855.
[17] D. D. Perrin, *Austral. J. Chem.*, 1964, **17**, 484.

reaction between the protonated substrate and water, as was observed for imidazole, takes place under such conditions.

The introduction of an alkyl group in the 1-position should make 2-H slightly less acidic and result in a result would be expected if the rate-determining step is between the protonated substrate and the hydroxide ion. The results in Table 2 therefore lend support to the latter viewpoint. The 2—3-fold acceleration in rate on alkyl substitution is in fact similar to that observed by Zatsepina [6] using EtOD as the solvent.

Although it has not been possible to investigate the effects of substituents on the pK_a of 2-H the results for N-3 protonation and N-1 ionisation are broadly as expected.

The insertion of two methyl groups, one on either side of 2-H, to form the 1,3-dimethylbenzimidazolium ion makes this hydrogen extremely labile as is also the case in the corresponding imidazolium ion.[18] These compounds may be regarded as analogues of the N-3 protonated cations and the value of k_2 ($3{\cdot}2 \pm 0{\cdot}2 \times 10^5$ l mol^{-1} s^{-1}) for the 1,3-dimethylbenzimidazolium ion, *ca.* 40 times faster than for benzimidazolium itself, is in line with the suggested mechanism.

Although the ionisation of carbon acids such as ketones and nitro-compounds are catalysed by bases in general, several studies [19,20] on heterocyclic compounds suggest that this is no longer the case and catalysis by bases other than hydroxide ion is insignificant. Our results at pH 4·43 (Table 3) for the 1,3-dimethylbenzimidazolium ion show that k_2 is virtually constant; the AcO^- : OH^- ratio was changed by a factor of 5 so that only the hydroxide ion acts as catalyst and the Brönsted exponent β for the reaction must be unity.

Recent work [21,22] has shown that in some reactions, *e.g.* the ionisation of nitro-compounds, where considerable solvent and structural reorganisation occurs, the exponent β may not be an accurate criterion of transition state structure. The reactions considered here however are very different in that the negative charge is localised and little reorganisation is necessary so that there is no reason to suppose that the β value of unity indicates anything other than a very product-like transition state. Although no primary hydrogen isotope effects have been measured in the present work the small values that have been obtained for similar systems (k_H/k_T in the range 1—5 for benzothiazole [6] and various thiazolium salts [23,24]) suggest that they are determined by equilibrium rather than kinetic factors. On this basis the protonation of the 1,3-dimethylbenzimidazolium ylide by water should be diffusion-controlled (10^{10}—10^{11} s^{-1}) leading to an estimated pK_a of between 19 and 20 at 85° for 2-H which compares with 16—18 at 30° for the same hydrogen in benzylbenzothiazolium bromide.[23] These ylides are only a few pK units more basic than the hydroxide ion yet they react with water at a diffusion-controlled rate. Such behaviour resembles more closely that of oxygen rather than carbon acids.

TABLE 1

Rate–pH data for [2-^{3}H]benzimidazole (1a), [2-^{3}H]-1-methylbenzimidazole (1b), and [2-^{3}H]-1-ethylbenzimidazole (1c)

	Compound (1a)		Compound (1b)		Compound (1c)	
pH (at 20°)	10^5k_{obs}/ s^{-1}	Rel. rate	10^5k_{obs}/ s^{-1}	Rel. rate	10^5k_{obs}/ s^{-1}	Rel. rate
13·00	33·2	0·42	249			
12·70	45·6	0·58				
12·30	63·3	0·80				
12·00	70·4	0·89	245	1·0		
11·00	76·8	0·98				
7·00	78·7	1·00	243		215	1·0
5·55			213	0·87	200	0·93
5·45	70·7	0·90				
4·90	54·8	0·70	171	0·70	145	0·67
4·65			113	0·46	105	0·49
4·59	38·7	0·49				
4·18	22·2	0·28				
3·90			35	0·14	36	0·17
3·71	12·5	0·16				
3·10			8·2	0·034		
2·00			0·65	0·0027		

TABLE 2

Rates of detritiation at 85° in neutral solution and equilibrium data *

Compound	10^5k_{obs}/s^{-1}	pK_a	pK_a'
Benzimidazole	78·7	5·53	13·2
1-Methylbenzimadazole	246	5·55	
1-Ethylbenzimidazole	215	5·57	
1-Isopropylbenzimidazole	163		
5,6-Dichlorobenzimidazole	19·1		11·3
4,5,6-Trichlorobenzimidazole	7·50		10·6
4,5,6,7-Tetrachlorobenzimidazole	2·93		9·33

* In H_2O at 25°.

TABLE 3

Rates of detritiation of [2-^{3}H]-1,3-dimethylbenzimidazolium bromide in aqueous buffers at 85°, μ = 0·1M

pH at 20°	10^5k_{obs}/s^{-1}	$10^{-5}k_2$/ l mol^{-1} s^{-1}
3·35	25·4	3·58
3·70	47·8	3·02
4·07	113	3·05
4·25	163	2·90
4·43	267	3·14
4·43	261	3·07
4·43	268	3·15
4·50	358	3·58
4·56	390	3·39

lowering of k_{obs} if the mechanism involves proton abstraction by the water molecule from the neutral substrate. Similarly the insertion of several chlorine atoms in the benzene ring should increase k_{obs} if this mechanism is operative. On the other hand the opposite

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[19] J. A. Zoltewicz and L. S. Helmick, *J. Amer. Chem. Soc.*, 1970, **92**, 7547.
[20] J. A. Zoltewicz, C. L. Smith, and G. M. Kauffman, *J. Heterocyclic Chem.*, 1971, **8**, 337.
[21] F. G. Bordwell, W. J. Boyle, jun., J. A. Hautala, and K. C. Yee, *J. Amer. Chem. Soc.*, 1969, **91**, 4002.
[22] F. G. Bordwell, W. J. Boyle, jun., and K. C. Yee, *J. Amer. Chem. Soc.*, 1970, **92**, 5926.
[23] D. S. Kemp and J. T. O'Brien, *J. Amer. Chem. Soc.*, 1970, **92**, 2554.
[24] W. Hafferl, R. Lundin, and L. L. Ingraham, *Biochemistry*, 1963, **2**, 1298.

Finally, mention must be made of the fact that many heterocyclic compounds labelled with tritium are used in chemical and biochemical investigations where inadvertently an opportunity may exist for the tritium to exchange with the solvent.[25] Our results show that difficulties which may arise with imidazoles can be reduced by employing acidic media (pH **1**—**3**). Storage under alkaline conditions is not recommended.

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[25] E. A. Evans, H. C. Sheppard, and J. C. Turner, *J. Labelled Compounds*, 1970, **6**, 76.