**932.** *The Polarography of Some Simple Pyrazine Derivatives.*

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Simple pyrazines have been found to give rise to three polarographic waves. The first wave is due to a two-electron irreversible reduction, probably to a dihydropyrazine. The variation with pH of the half-wave potential indicates a pK_a of 3·6 for tetramethylpyrazine, but lower values for the other pyrazines investigated. This has been confirmed by the spectrophotometric determination of the dissociation constants. The other pyrazine waves are not simple diffusion-controlled waves.

DURING investigations of the products of the reaction of molasses with ammonia, it was found [1] that polarographically active substances were formed which were subsequently identified as simple pyrazines. Little has been reported on the polarography of pyrazines,[2,3] and the polarographic behaviour of some simple derivatives has therefore been examined.

The pyrazines investigated gave polarographic reduction waves over a wide range of pH. In pure solutions there were very pronounced maxima but these were easily eliminated by the addition of gelatin. A typical polarogram is shown in the Figure for tetramethylpyrazine
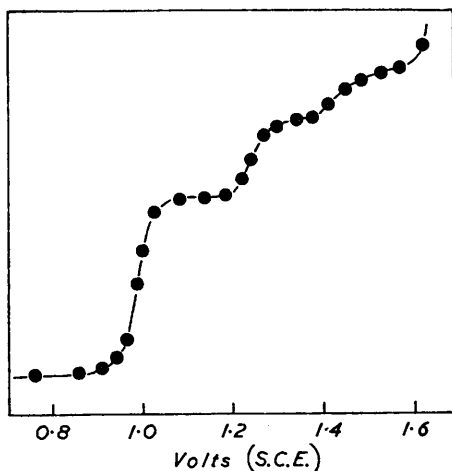
Polarogram of tetramethylpyrazine in M-phosphate buffer of pH 6·9 containing 0·004% of gelatin.

in a phosphate buffer of pH 6·9. Three waves can be distinguished. The first wave, *i.e.* that at the most positive potential, remained unchanged in height over a wide range of pH for a given pyrazine concentration. The heights of the second and the third wave were dependent on the pH of the solution. At pH about 1, the two waves became merged and the total wave height was roughly equal to that of the first pyrazine wave. As the pH was increased, the heights of the second and the third wave decreased until by pH 9 they had disappeared. The other pyrazines investigated behaved similarly.

The First Pyrazine Wave.—The height of the first pyrazine wave was proportional to the pyrazine concentration (0·1—10 mM) for all the pyrazines investigated, and the limiting currents were not affected by the gelatin concentration, below 0·05%, when the pH of the solutions was below 7. In more alkaline solutions the wave became badly shaped and the limiting current decreased slightly with increasing gelatin concentration. The wave height was approximately proportional to the square root of the height of the mercury reservoir so that it is a normal diffusion-controlled wave.

[1] Wiggins and Wise, *Chem. and Ind.*, 1955, 656.
[2] Bonito, *Pontif. Acad. Sci. Acta*, 1945, **9**, 65.
[3] Mader and Frediani, *Analyt. Chem.*, 1948, **20**, 1199; Rickes, Trenner, Conn, and Keresztesy, *J. Amer. Chem. Soc.*, 1947, **69**, 2751; Allen, Pasternak, and Seaman, *ibid.*, 1952, **74**, 3264.

Values for the diffusion-current constant, I_d, as defined by Lingane,[4] are given in Table 1.

TABLE 1. *Diffusion-current constants of pyrazine derivatives.*

Pyrazine	M	0·5n-HCl	m-Phosphate buffer, pH 3·06	m-Acetate buffer, pH 4·39
2-Methyl-	94	3·83	3·73	3·59
2 : 5-Dimethyl-	108	3·70	3·46	3·42
Tetramethyl-	136	3·45	3·26	3·16
5-(D-*arabo*Tetrahydroxybutyl)-2-methyl-	213	2·98	2·89	2·83
2 : 5-Bis-(D-*arabo*tetrahydroxybutyl)- ...	320	3·19	2·80	2·71

The number of electrons involved in the reaction at the dropping-mercury electrode was established as two by using the coulometric technique.[5]

Logarithmic analyses of the waves did not give the slopes (0·030) expected for an ordinary two-electron reduction, and the slope was dependent on pH, as shown for example by the results for tetramethylpyrazine in Table 2. It is therefore evident that the first

TABLE 2. *Variation of slope of log plot with* pH *for tetramethylpyrazine.*

pH	1·3	2·7	3·3	4·7	6·2	7·3
Slope of log plot	0·049	0·049	0·046	0·042	0·033	0·031

pyrazine wave represents an irreversible two-electron reduction. The most likely reaction product is a dihydropyrazine.

Dissociation Constants of the Pyrazines.—Four of the pyrazine derivatives investigated gave a linear relation between half-wave potential and pH of the form $-E_{\frac{1}{2}} = E_0 + 0.085\text{pH}$. Values of E_0 for the different pyrazines were : 2-methyl, 0·32 ; 2 : 5-dimethyl, 0·35 ; 5-(D-*arabo*tetrahydroxybutyl)-2-methyl, 0·34 ; 2 : 5-bis-(D-*arabo*tetrahydroxybutyl), 0·34. The half-wave potentials of these pyrazines were therefore almost identical at all pH values. Tetramethylpyrazine behaved differently, and its half-wave potential can be expresssed as $-E_{\frac{1}{2}} = 0.48 + 0.059\text{pH}$ below and as $-E_{\frac{1}{2}} = 0.38 + 0.087\text{pH}$ above pH 3·6.

The variation of the half-wave potential of pyrazines with pH indicates that hydrogen ions are involved in the electrode reduction, as would be expected if the reduction product is a dihydropyrazine, although the theoretical slope of 0·059 is only found for tetramethyl-pyrazine below pH 3·6. The results for this pyrazine suggest that it has a pK_a of 3·6 ; below pH 3·6 the salt is reduced and above pH 3·6 the free base. The other pyrazines did not appear to form salts at a pH within the range of the polarographic investigation.

The dissociation constants of the pyrazines investigated have not been recorded, although that of pyrazine itself is known.[6] The ultraviolet absorption spectra of pyrazine in sulphuric acid solutions [7] suggested that the dissociation constants could be measured spectrophotometrically,[8] and it was in fact found that the spectra of the salt form and of the free base were sufficiently different. As expected,[7] the absorption peak of the former was always at a higher wavelength than that of the latter. The wavelengths of the peaks and pK_a values are given in Table 3, which shows that the pyrazines are very weak bases. The pK_a is increased by the successive introduction of methyl groups, but the tetrahydroxybutyl side-chain has no influence. Tetramethylpyrazine is the only one investigated having a pK_a within the region where an inflexion is evident in the curve connecting half-wave potential with pH. The value of the dissociation constant of tetramethylpyrazine obtained spectro-photometrically agrees well with the value suggested by the polarographic measurements.

The spectrum of 3 : 6-di*iso*butyl-2-hydroxypyrazine was also determined. As will be seen from the absorption peaks, this compound differs markedly from the other pyrazines. Moreover, there was a further variation in the spectra for alkaline solutions ; this difference was not large but corresponded to an ionisation at pH 10, probably due to the ionisation of

[4] Lingane, *Ind. Eng. Chem. Anal.*, 1954, **15**, 588.
[5] Wise, unpublished results.
[6] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.
[7] Halverson and Hirt, *J. Chem. Phys.*, 1951, **19**, 711.
[8] Beale, *J.*, 1954, 4494.

the hydroxy-group. There is, however, the possibility of tautomerism in 2-hydroxy-pyrazines, as has been found for the analogous pyrimidine and pyridazine derivatives.[9,10]

The Other Pyrazine Waves.—These were not investigated in great detail as they were found not to be simple diffusion-controlled waves. Their heights were not accurately proportional to pyrazine concentration and were affected by the concentration of gelatin. In solutions of higher pH, where the wave heights were comparatively small, they became

TABLE 3. *Absorption peaks and* pK_a *values of pyrazines.*

Pyrazine	Salt, $m\mu$	Free base, $m\mu$	pK_a
Unsubstituted	—		0·6
2-Methyl-	280	270	1·4
2 : 5-Dimethyl-	290	275	1·9
Tetramethyl-	300	double peak	3·7
5-(D-*arabo*Tetrahydroxybutyl)-2-methyl-	285	275	1·4
2 : 5-bis-(D-*arabo*Tetrahydroxybutyl)-	290	275	0·7
3 : 6-Di*iso*butyl-2-hydroxy-	350	330	1·7

TABLE 4. *Dependence of wave heights on mercury pressure.*

Pressure, cm.	66	47	36	31
First wave, μA	4·7	3·8	3·4	3·0
Second wave, μA	1·9	1·8	1·8	1·7
Third wave, μA	1·0	1·0	0·9	0·9

independent of the height of the mercury reservoir. This is illustrated (Table 4) for a solution which contains $5·3 \times 10^{-4}$M-tetramethylpyrazine and 0·004% of gelatin in M-phosphate buffer of pH 6·9. This behaviour is typical of waves controlled kinetically;[11] such waves are often found for heterocyclic nitrogen compounds.

EXPERIMENTAL

2-Methyl- and 2 : 5-dimethyl-pyrazine were purified *via* the picrate from samples kindly donated by the Wyandott Chemicals Corporation. Tetramethylpyrazine was prepared by the reaction of ammonia with β-bromolævulic acid,[12] and 2 : 5-bis-(D-*arabo*tetrahydroxybutyl)-pyrazine from fructose and methanolic ammonia.[13] The specimen of 5-(D-*arabo*tetrahydroxybutyl)-2-methyl pyrazine, m. p. 202°, was isolated from the reaction products of invert sugar and ammonia (Davison and Wiggins, unpublished results). 3 : 6-Di*iso*butyl-2-hydroxypyrazine was kindly given by Professor F. S. Spring.

All measurements were carried out with a Radiometer type PO3h polarograph at $25 \pm 0·5°$. The capillary used had a value for $m^{2/3}t^{1/6}$ of 2·75 at the top of the first pyrazine wave in 0·5N-hydrochloric acid. Half-wave potentials were measured against a saturated calomel electrode by recording the polarograms manually with a mercury-pool anode and measuring the potential of the dropping-mercury electrode against a standard calomel electrode with a Cambridge pH meter. The gelatin solution used was freshly prepared each day.

Dissociation constants were measured with a Hilger Uvispec spectrophotometer. The spectra of the salt forms were measured in 2N-hydrochloric acid and of the free bases in water and dilute sodium hydroxide. The spectra when both forms were present were recorded in hydrochloric acid solutions of pH approximately equal to the pK_a of the pyrazine, except for tetramethylpyrazine when a 2mM-acetate buffer was used. The pH of the solutions was measured with a Cambridge pH meter. The pK_a values are not corrected for salt effects.

[9] Marshall and Walker, *J.*, 1951, 1004.
[10] Overend, Turton, and Wiggins, *J.* 1950, 3505.
[11] Kolthoff and Lingane, " Polarography," Interscience, New York, 1952, p. 274.
[12] Wolff, *Ber.*, 1887, **20**, 428.
[13] de Bruyn and van Ekenstein, *Rec. Trav. chim.*, 1899, **18**, 76, 80.