POLAROGRAPHIC REDUCTION OF 3-O-METHYLPYRIDOXAL 5'-PHOSPHATE

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The polarographic reduction of 3-O-methyl-pyridoxal 5'-phosphate has been studied as a function of pH. The reduction of the aldehyde group by a two electrons process occurs in all conditions studied. The rate of interconversion between the free and hydrate forms of the carbonyl group has been computed. This reaction is acid–base catalyzed. The other reactions (proton transfers) that precede the electrodic reaction are very much faster. The results are compared with those previously obtained for pyridoxal 5'-phosphate.

The polarographic behaviour of pyridoxal 5'-phosphate in nonaqueous and aqueous solutions at the dropping mercury electrode has been studied by several researchers[1-4]. It was shown that the rate of interconversion between free and hydrated forms of the carbonyl group is very fast[3]. However, the dehydration rates for other pyridine aldehydes[5-9] (even, pyridoxal[2]) are slower and they govern the heights of the polarographic waves. One or more of the protonable groups of pyridoxal 5'-phosphate could be implied in the high dehydration rate found for this molecule, for example, by an intramolecular acid–base catalysis. The 3-hydroxy group is the most promissing candidate for this type of reaction. Therefore, we have studied the polarographic behaviour of 3-O-methylpyridoxal 5'-phosphate in this paper.

EXPERIMENTAL

Polarographic current-voltage curves were performed at 22°C in buffered solutions. The measurements below pH 1 were carried out using H_2SO_4 solutions by means of the Hammett function[10]. Unless otherwise specified the buffers used were 0·20M sodium acetate–acetic acid, monosodium phosphate–disodium phosphate and sodium bicarbonate–sodium hydroxide depending on the requiered pH. The saturated calomelan electrode (S.C.E.) was always used as reference electrode. The dropping mercury electrode used had the following properties: $m = 1{\cdot}4$ mg s^{-1} and $t = 4{\cdot}4$ s, determinated at zero potential.

The 3-O-methylpyridoxal 5'-phosphate was prepared from pyridoxine by a new method in four steps[11]. The UV and ^{1}H-NMR spectra coincide with those published in the literature[12-14]. Its concentrations were measured by dilution of a recently prepared stock solution with 0·1M-HCl

and measuring the absorption at 282 nm. An absorbance index of 7100 was used[12]. The dehydration and catalytic rate constants were calculated using the Koutecky theoretical treatment[15].

RESULTS

The polarographic reduction of 3-O-methylpyridoxal 5'-phosphate has been studied in aqueous solution as a function of pH. A single diffusion wave (i_1) appears at

TABLE I

Concentration Dependence of the Intensity and Half-Wave Potential of 3-O-Methylpyridoxal 5'-Phosphate at pH 6·94

c, mM	0·0637	0·1272	0·1686	0·2250	0·292	0·331
$-E_{1/2}$, mV	688	693	694	697	702	705
i, μA	0·273	0·531	0·741	0·980	1·260	1·414

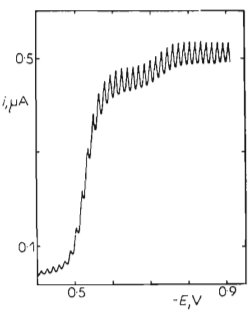

FIG. 1

Polarogram of 3-O-Methylpyridoxal 5'--Phosphate at pH 4·75

The potencials are referred to S.C.E. $c = 0·090$ mM.

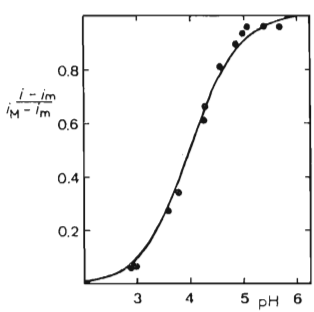

FIG. 2

Variation of the wave Intensity of 3-O--Methylpyridoxal 5'-Phosphate with pH

The minimum (i_m) and maximum (i_M) intensity values were taken as $i_m = 0·075$ μA and $i_M = 0·153$ μA. The concentration was 0·036 mM and the buffer system was acetic acid–sodium acetate 0·2M. The solid line is the theoretical for the dissociation of a monoprotic acid with $pK = 4·0$.

neutral pH with a diffusion current constant of 2·7 (Table I), very similar to that obtained for pyridoxal 5'-phosphate[3], corresponding to a two electrons process. Its half-wave potential changes slightly and linearly with the concentration of the electroactive substance (Table I). A very small adsorption wave is observed at more

TABLE II

Influence of the Buffer on the Wave Intensity of 3-O-Methylpyridoxal 5'-Phosphate at pH 6, $c = 0{\cdot}036$ mM

Buffer used	phosphate	acetate	acetate	acetate
Concentration	0·2*B*	0·2M	0·05M	0·01M
Wave intensity, μA	0·153	0·147	0·133	0·122

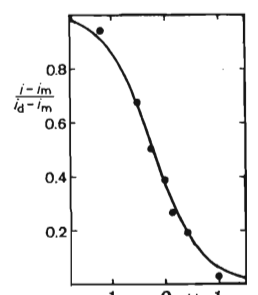

FIG. 3

Variation of the Wave Intensity of 3-O--Methylpyridoxal 5'-Phosphate with the Hammett Function

H_2O–H_2SO_4 mixtures were used to obtain the indicate H_0 values. The values of i_d and i_m were 0·40 and 0·15 μA respectively. $c =$ = 0·095 mM. The solid line is the theoretical curve for the dissociation of a monoprotic acid with a $pK = -0{\cdot}2$.

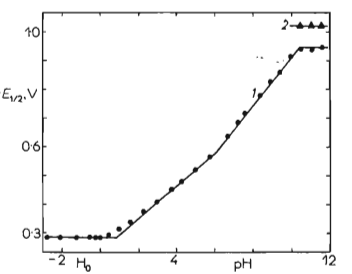

FIG. 4

pH-Dependence of the Half-Wave Potentials *vs* S.C.E. for 3-O-Methylpyridoxal 5'-Phosphate

The experimental values for i_1 *1* and i_2 *2* are plotted *vs* pH or Hammett function, $c = 0{\cdot}10$ mM.

negative potentials (Fig. 1) when the concentration is very low and for this reason we shall not consider it any more along this paper.

The wave intensity decreases with decreasing pH to a minimum value (i_m) around pH 2, according to the dissociation curve for a monoprotic acid with an apparent pK of 4·0 (Fig. 2). The value observed at pH 6 (i_M) depends on the concentration and type of buffer used (Table II) and the current shows a slight kinetic character at this pH. This character increases with decreasing pH and i_m is practically independent of the mercury height. This behaviour is similar to that of many other pyridinecarboxaldehydes[2,5-9] in which a slow dehydration reaction of the carbonyl group is combined with an acid–base equilibrium. The pK for the deprotonation of the pyridinium nitrogen, pK = 4·0, (Fig. 2) is close to that measured at low ionic strength by spectrophotometric titration, pK = 4·15, ref.[12].

The total reduction current increases with increasing concentration of a strong acid (Fig. 3), like with other pyridinecarboxaldehydes[2,5-9], due to the fact that the dehydration reaction is acid–base catalyzed. Assuming $k = k_0 + k_1 [H^+]$ we computed numerical values for k_0 and k_1 from the experimental data given in Fig. 3 and Table III. The equation $i/(i_d - i) = 0{\cdot}487 + 1{\cdot}053\,[H^+]$ is obtained by the least square adjustment with a correlation index of 0·999. Then $k_0 = 0{\cdot}487^2 \,.\, 9{\cdot}5/(0{\cdot}886^{-2}\sqrt{4{\cdot}8})^2 = 0{\cdot}60\ s^{-1}$ and $k_1 = 1{\cdot}053^2 \,.\, 9{\cdot}5/(0{\cdot}886^2\sqrt{4{\cdot}8}) = 2{\cdot}8\ s^{-1}\ dm^3\ .\ mol^{-1}$, where we have used a drop time of 4·8 s and the equilibrium constant for the dehydration reaction found by Bazhulina and coworkers[14] ($K = [H_2O]/q_1 = 9{\cdot}5$). The value of k_0 is close to that measured by T-jump experiments for this compound ($k_0 = 0{\cdot}65\ s^{-1}$). Larger differences are instead observed between their results for k_1, $30\ s^{-1}\ dm^3\ mol^{-1}$, and ours, $2{\cdot}8\ s^{-1}\ dm^3\ mol^{-1}$. We would like to note that the catalytic constants obtained by these authors were higher that those obtained by

TABLE III

Calculation of the Dehydration Rate Constants for 3-O-Methylpyridoxal 5'-Phosphate

H_0[a]	$[H^+]$	$10i$, μA[a]	$i/(i_d - i)$[b]
1·00	0·10	1·55	0·633
0·40	0·40	1·98	0·980
0·15	0·71	2·16	1·174
0·00	1·00	2·48	1·632
−0·25	1·78	2·75	2·200
−0·50	3·16	3·17	3·819
−1·20	15·85	3·78	17·182

[a] The H_0 and i values were taken from Fig. 3; [b] $i_d = 0{\cdot}40$ μA.

other authors (for other compounds)[16,17], and their procedure has been criticized[17-18]. Only with irradiated solutions the wave at pH below 2 is splitted in two waves of equal intensity, as it was described for pyridoxal[2].

At pH values higher than 9 a new wave (i_2) appears with more negative half-wave potential (Fig. 4) which is pH independent. The intensity of this new wave increases with increasing pH at the expense of wave i_1, keeping the total intensity constant. The height of i_1 decreases in the form of a dissociation curve for a monoprotic acid (Fig. 5) with a pK' value of 10·4. The same value for pK' can be obtained from the break of the $E_{1/2}$–pH plot (Fig. 4). This pK' is very near to the one found for pyridoxal 5'-phosphate[3] although in the present case there is not another additional wave and consequently the second pK' is missing.

The pH dependence of the half-wave potentials of i_1 and i_2 (Fig. 4) practically coincides with that obtained for pyridoxal 5'-phosphate[4]. In the study of this compound a high effect of the N,N'-dimethylformamide and tetramethylammonium cations on the wave intensities was found[4]. We found a similar behaviour with 3-O-methylpyridoxal 5'-phosphate, except that now the results for $N(CH_3)_4^{(+)}$ are better fitted for the dissociation curve of a monoprotic acid (Fig. 6) than the curve corresponding to a diprotic acid as in the case of pyridoxal 5'-phosphate[4].

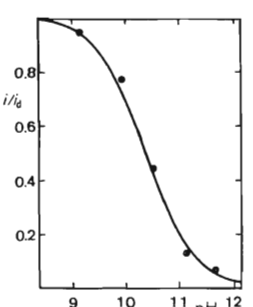

FIG. 5

pH-Dependence of the Wave-Height of Limiting Current i_1 for 3-O-Methylpyridoxal 5'-Phosphate

The solid curve is the theoretical one for the dissociation of a monoprotic acid with pK = 10·4, c = 0·10 mM.

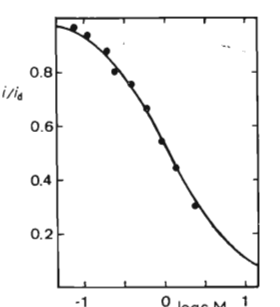

FIG. 6

Variation of the Relative Wave-Height of i_1 for 3-O-Methylpyridoxal 5'-Phosphate with $N(CH_3)_4Br^{(+)}$ Contents at pH 8·9

The theoretical curve for the dissociation of a monoprotic acid is also plotted, c = = 0·133 mM.

DISCUSSION

The most drastic difference in the polarographic reduction of pyridoxal 5'-phosphate and the same molecule with the 3-hydroxy group methylated is the absence of the most negative wave in the last case. This result implies that the form reduced in the third wave for pyridoxal 5'-phosphate (i_3) was the most deprotonated structure as it was already supposed[3]. Furthermore, the coincidence between the half-wave potentials for the wave i_1 measured for both compounds confirms our previous assumption that the form reduced in i_2 was the one corresponding to the structure with the phenolic group protonated[3]. However, the half-wave potential of this wave (i_2) is 0·13 V more negative for pyridoxal 5'-phosphate[4] (−1·15 V) than for the 3-O-methylpyridoxal 5'-phosphate (−1·02 V, Fig. 4). This difference is higher than the expected from the inductive effect of the H and CH_3 groups. The assumed hydrogen bond between the carbonyl and phenolic groups of pyridoxal 5'-phosphate[3] could explain this difference.

While the total reduction current of pyridoxal 5'-phosphate, corresponding to a two electrons process, is pH independent it shows great changes with pH for the 3-O-methylpyridoxal 5'-phosphate (Fig. 2 and 3). These changes can be explained by the existence of a slow dehydration reaction of the carbonyl group combined with the very fast reaction of protonation of the pyridinium nitrogen. The values obtained for the kinetic constant of the dehydration reaction are given under results, and they show that the reaction undergoes a general acid–base catalysis. The ^{1}H-NMR spectra of both compounds at pH lower that 4 indicate a signal for the aldehydic proton at 6·5 ppm (free aldehyde). We may conclude that the dehydration reaction of the carbonyl group will be faster when the 3-hydroxy group is not methylated, *i.e.* this OH group may catalyse intramolecularly the dehydration reaction.

The effect observed when either N,N'-dimethylformamide or tetramethylammonium cations are added to the solution, is very similar to that observed for pyridoxal 5'-phosphate and we do not observe any change in the 3-O-methylpyridoxal 5'-phosphate absorption spectrum by addition of these compounds at the maximum concentration given in the Fig. 6. A hypothetical complex between these chemicals could explain these results[4].

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