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Electroörganic Preparations

XII. Polarography and Reduction of Isonicotinic Hydrazide and Related Compounds

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Isonicotinic hydrazide is reduced polarographically in acid and neutral solution in two waves. The electrode reaction corresponding to the first wave is a reduction to isonicotinic amide. The second electrode reaction is in acid solution a reduction to pyridine-4-aldehyde which is partly protected against further reduction to γ-pyridyl carbinol by the formation of a non-reducible hydrate. In alkaline solution isoniazid gives an anodic wave and the corresponding electrode reaction is an oxidation to 1,2-diisonicotinoyl-hydrazine, nitrogen and varying amounts of isonicotinic acid. 1,2-Diisonicotinoyl-hydrazine is in mineral acid solution in a 6-electron reaction reduced to pyridine-4-aldehyde and ammonia.

Isonicotinic hydrazide (isoniazid) has previously [1,2] been investigated polarographically, but the nature of the electrode reactions has not been established. In the present work isoniazid and a few related compounds have been investigated polarographically and the electrode reactions studied by means of constant potential reductions and oxidations.

POLAROGRAPHIC INVESTIGATION

In Tables 1 and 2 are given the results from the polarographic investigation of isoniazid. In Table 1 are included the data of isonicotinic amide.

At the concentration used in the polarographic investigation, 0.3×10^{-3} M (Table 1), the height of the anodic wave of isoniazid corresponds to an electron consumption of four electrons per molecule. The half-wave potentials, which are somewhat dependent on the concentration, lie from pH 8 to 13.5 on a line with a slope of 0.077 V/pH.

The height of the cathodic wave is proportional to concentration up to at least 10^{-2} M, but this is not true for the anodic wave. In Table 2 is given the dependence of the ratio between the anodic i_A and the cathodic i_C limiting

Table 1. Limiting currents in μA and half-wave potentials *vs* S.C.E. of isoniazid and isonicotinic amide. Concentration 40 mg/l.

				Isoniazid				Isonicotinic	
		Anodic wave		1. wave		2. wave		amide	
Buffer	pH	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$
6 N HCl				2.64	0.50	2.42	0.57	3.30	0.57
2.5 N HCl				2.70	0.47_5	2.88	0.58	3.68	0.58
HCl—KCl	0.40			2.65	0.47_5	2.80	0.60_5	3.75	0.60_5
»	0.70			2.70	0.47_5	2.75	0.62_5	3.75	0.63
»	0.90			2.70	0.47_5	3.05	0.64	3.80	0.64
»	1.50			2.70	0.48	3.50	0.67	4.30	0.66_5
Glycine	2.10			2.70	0.53	4.00	0.70_5	5.00	0.70_5
Citrate	2.60			2.55	0.59	4.30	0.73	5.35	0.72
»	3.05			2.60	0.61	4.80	0.75_5	5.55	0.75
Acetate	4.25			2.70	0.72_5	5.35	0.86_5	6.65	0.86
»	4.75			2.75	0.78	5.30	0.91	6.50	0.91
Succinate	5.05			2.70	0.79_5	5.40	0.94	6.70	0.93
Phosphate	6.15			2.80	0.93	5.20	1.03_5	6.60	1.03
»	6.60			2.70	0.96_5	5.45	1.07	6.80	1.07
»	7.10			2.80	1.01	4.20	1.10_5	5.70	1.10_5
Borate	8.25	−5.0	-0.07_5	2.70	1.12_5	2.70	1.18_5	3.90	1.17
»	9.00	−5.3	−0.02			5.50	1.20	3.80	1.21_5
»	9.70	−5.5	0.02			5.30	1.23	3.70	1.24_5
Phosphate	11.05	−4.9	0.12			5.05	1.30_5	3.70	1.30
»	11.80	−4.9	0.19			4.20	1.35	3.70	1.32
0.02 N KOH	12.5	−4.9	0.24			3.40	1.40		
0.1 N »	13.0	−5.0	0.28			2.8	1.45	3.90	1.34_5
0.5 N »	13.5	−5.0	0.30			2.8	1.46		

Table 2. Dependence of the ratio between anodic and cathodic limiting currents on the concentration of isoniazid at different pH.

Concentration mg isoniazid/l	0.5 M borate buffer pH = 9.0	0.5 M phosphate buffer pH = 11.2	0.2 M KOH
20	1.11	1.08	1.90
40	1.12	1.13	1.94
80	1.09	1.09	1.90
200	1.05	0.95	1.90
400	0.65	0.86	1.92
600	0.51	0.84	1.94
800	0.44	0.80	1.91
1000	0.39	0.74	1.82
2000	0.20	0.56	1.63

current at pH 9, 11, and 13. The ratio i_A/i_C falls much faster with concentration at lower than at higher pH. The lower ratio i_A/i_C at higher concentrations is consistent with the finding that in a preparative oxidation in a phosphate buffer pH 11.2 the reaction consumed 2 electrons per molecule.

From the height of the first cathodic wave the electrode reaction is expected to consume two electrons per molecule, and this is also found in a macroscale reduction. The half-wave potentials are independent of pH in strongly acid solution up to pH 1.5. Between pH 1.5 and 7.5 the half-wave potentials become

0.095 V more negative per pH. Around pH 9 the first wave merges with the second one.

The second wave of isonicotinic hydrazide and the wave of isonicotinic amide are very much alike both with respect to half-wave potentials and limiting currents. In mineral acid solution the wave-height corresponds to two electrons per molecule, but between pH 1 and 4 it grows to the height of a four-electron wave. Above pH 7 the wave-height decreases again. The wave-height of isonicotinic amide decreases at higher pH to the height of a two-electron wave, but for isoniazid the picture is more complicated, as the first and the second wave merge in the same pH-region. The combined wave of isoniazid has from pH 9 to 11.5 the height of a four-electron wave.

The pH-dependence of the half-wave potentials of the second wave of isoniazid can up to pH 7 be approximated by two lines: one from pH −0.5 to 3 with a slope of 0.055 V/pH and another one from pH 3 to 7 with a slope of 0.085 V/pH. Between pH 9 and 11.5 the half-wave potentials of the combined wave become 0.057 V more negative per pH.

Between pH 11.5 and 13 the wave-height of isoniazid diminishes to the height of a two-electron wave. In more strongly alkaline solution the wave-height remains constant up to an alkaline strength of at least 0.5 N potassium hydroxide.

1-Isonicotinoyl-2,2-dimethyl-hydrazine behaves polarographically very much like isoniazid, but no anodic wave is found.

Table 3. Limiting currents in μA and half-wave potentials *vs* S.C.E. of 1-isonicotinoyl-2-phenyl-hydrazine and 1,2-diisonicotinoyl-hydrazine. Concentration 40 mg/l.

	1-isonicotinoyl-2-phenyl-hydrazine							1,2-diisonicotinoyl-hydrazine.		
		Anodic wave		1. wave		2. wave				
Buffer	pH	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$	i_l	$-E_{\frac{1}{2}}$	$-E_{\frac{1}{2}}$
6 N HCl				1.00	0.45	1.02	0.58	2.55	0.53_5	
2.5 N HCl				1.05	0.46	1.15	0.59_5	2.55	0.55	
HCl−KCl	0.40			1.10	0.49_5	1.15	0.60_5	2.60	0.58_5	
»	0.70			1.10	0.52_5	1.20	0.62_5	2.64	0.59	
»	0.90			1.10	0.53_5	1.20	0.64_5	2.76	0.60_5	
»	1.50			1.05	0.57	1.40	0.67_5	3.12	0.61_5	0.69
Glycine	2.10			1.05	0.61	1.60	0.71	3.32	0.63	0.71
Citrate	2.60			1.05	0.63	1.70	0.72	3.35	0.66	0.73
»	3.05			1.10	0.66_5	1.80	0.75_5	3.40	0.69	0.76
Acetate	4.25			1.10	0.74	2.10	0.86	3.52	0.76	0.87
»	4.75			1.05	0.77_5	2.05	0.90_5	4.00	0.80	0.91
Succinate	5.05			1.05	0.80_5	2.15	0.93_5	4.05	0.82	0.94
Phosphate	6.15			1.10	0.89	2.15	1.02_5	4.00	0.91	1.03
»	6.60			1.05	0.93	2.00	1.07	3.72	0.94	1.07
»	7.15			1.10	0.96_5	1.70	1.10_5	3.44	0.98	1.11
Borate	8.25	−1.9	0.00	1.05	1.04	1.05	1.17	2.64	1.07	1.18
»	9.00	−1.9	0.03	1.05	1.11	1.00	1.21	2.64	1.10	1.21
»	9.70	−1.9	0.06	1.00	1.15	1.00	1.23	2.44	1.23	
Phosphate	11.05	−1.8	0.14			1.85	1.27_5	1.60	1.34	
»	11.80	−1.7	0.17			1.75	1.31_5	1.40	1.38	
0.1 N KOH		−1.9	0.24			1.90	1.44	1.35	1.46	

The polarographic behaviour of 1-isonicotinoyl-2-phenylhydrazine (Table 3) resembles that of isoniazid, but some differences exist. The half-wave potentials of the first cathodic wave are not independent of pH at low pH-values, but can be approximated by a single line from pH 0 to 9 with a slope of 0.072 V/pH. Furthermore, the wave-height does not diminish in alkaline solution up to pH 13.

1-Isonicotinoyl-2-methyl-2-phenyl-hydrazine resembles polarographically 1-isonicotinoyl-2-phenyl-hydrazine, but no anodic wave is found.

Some of the compounds give rise to a maximum on the first cathodic wave. This is especially pronounced in the reduction of 1,2-diisonicotinoyl-hydrazine. The height of the first wave depends somewhat on the nature of the buffer, wherefore in Table 3 are given only the sum of the limiting currents of the cathodic waves together with the half-wave potentials of these waves. The polarographic behaviour of the compound resembles that of the above mentioned compounds. No anodic wave is found.

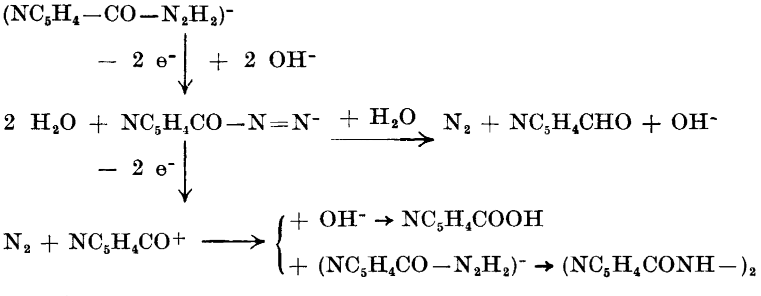
MACROSCALE REACTIONS

The course of the anodic reaction of isoniazid depends on pH. In a phosphate buffer pH 11.2 the main product of the two-electron oxidation was 1,2-(diisonicotinoyl)-hydrazine, which was isolated. Besides, a small amount of pyridine-4-aldehyde could be detected polarographically.

In 0.2 M potassium hydroxide containing 1 M potassium chloride the oxidation required 2.9 electrons per molecule, and about 45 % isonicotinic acid was formed besides about 55 % 1,2-diisonicotinoyl-hydrazine. It was noteworthy that the current density could be more than five times higher in 0.2 M KOH than in phosphate buffer pH 11.2, although the same concentrations of isoniazid were used.

The isonicotinic acid found in the reaction mixture was not formed by hydrolysis of isoniazid or 1,2-diisonicotinoyl-hydrazine. The stability of these compounds was tested under conditions similar to those used during the oxidation, and no hydrolysis products were found.

The anodic reaction of isoniazid in alkaline solution can be formulated as:



$$\begin{tikzcd}
(NC_5H_4{-}CO{-}N_2H_2)^- \arrow[d, "-\,2\,e^-"', "+\,2\,OH^-"] & \\
2\,H_2O + NC_5H_4CO{-}N{=}N^- \arrow[r, "+\,H_2O"] \arrow[d, "-\,2\,e^-"'] & N_2 + NC_5H_4CHO + OH^- \\
N_2 + NC_5H_4CO^+ \arrow[r] & \left\{\begin{array}{l} +\ OH^- \rightarrow NC_5H_4COOH \\ +\ (NC_5H_4CO{-}N_2H_2)^- \rightarrow (NC_5H_4CONH{-})_2 \end{array}\right.
\end{tikzcd}$$

The anodic wave of 1-isonicotinoyl-2-phenyl-hydrazine has the height of a four-electron wave at low concentrations. In a preparative oxidation in 0.2 N potassium hydroxide $n = 2.5$ was found, and the only identified product was isonicotinic acid. No 1,2-diisonicotinoyl-hydrazine could be detected. A dark-grey precipitate containing mercury was formed. It resembled the precipitate formed when mercuric chloride is added to a solution of phenylhydrazine. Besides, a yellow, ether-soluble compound was formed. In the beginning of the oxidation a compound, which was reduced at a potential 0.5 V less negative than 1-isonicotinoyl-2-phenyl-hydrazine, was formed, but it disappeared gradually near the end of the reaction. The reaction might be formulated as:

$$\begin{tikzcd}
NC_5H_4CO-N^--NH-C_6H_5 \arrow[r, "-\,2\,e^-", "+\,OH^-"'] & NC_5H_4-CO-N=N-C_6H_5 + H_2O \arrow[d] & \\
& NC_5H_4CO^+ \arrow[d, "+"', "OH^-"] & +\ C_6H_5-N=N^- \arrow[d] \\
& NC_5H_4COOH & \text{further oxidation or reaction with } Hg^{++}
\end{tikzcd}$$

1-Isonicotinoyl-2,2-dimethyl-hydrazine, 1-isonicotinoyl-2-methyl-2-phenyl-hydrazine and 1,2-diisonicotinoyl-hydrazine are not oxidized at the anode.

The first cathodic wave of isoniazid was found to be caused by a reductive splitting of the nitrogen-nitrogen bond. In strongly acid solution, where the half-wave potentials are independent of pH, the reaction proceeds according to:

$$\begin{aligned} HN^+C_5H_4-CO-NH-NH_3^+ + 2\ e^- &\rightarrow HN^+C_5H_4CO-NH^- + NH_3 \\ + 2\ H^+ &\rightarrow HN^+C_5H_4CO-NH_2 + NH_4^+ \end{aligned}$$

A similar reaction was found to cause the first cathodic wave of 1-isonicotinoyl-2,2-dimethyl-hydrazine, 1-isonicotinoyl-2-phenyl-hydrazine, and 1-isonicotinoyl-2-methyl-2-phenyl-hydrazine. The half-wave potentials of the compounds containing a phenyl group are not independent of pH in acid solution above pH 0.

The second cathodic wave of isoniazid is caused by the reduction of the isonicotinic amide formed in the first reduction. Isonicotinic amide is reduced in acid solution in the following way:

$$HN^+C_5H_4-CO-NH_2 + 2\ e^- + 3\ H^+ \rightarrow NH_4^+ + \left\{ \begin{array}{l} HN^+C_5H_4-CHO + H_2O \\ \qquad \downarrow\!\upharpoonleft \\ HN^+C_5H_4-CH(OH)_2 \end{array} \right.$$

If the reduction is allowed to proceed to completion at the potential necessary to reduce the amide, the free aldehyde is reduced to γ-pyridyl carbinol:

$$HN^+C_5H_4-CHO + 2\ e^- + 2\ H^+ \rightarrow HN^+C_5H_4-CH_2OH$$

Pyridine-4-aldehyde gives between pH 2 and 8 two one-electron waves. By using a suitable cathode potential, which is about 0.5 V less negative than that required for the reduction of isonicotinic amide, it is possible to reduce pyridine-4-aldehyde to a pinacol in good yield. The best conditions are by using a phosphate buffer with pH around 6.

A reduction in a phosphate buffer with pH 11 proved, that the four-electron wave of isoniazid found between pH 9 and 11.5 is caused by the following reaction, in which a dihydroisonicotinic amide and ammonia is formed:

$$NC_5H_4-CO-NH-NH_2 + 4\,e^- + 4\,H_2O \rightarrow HNC_5H_5-CO-NH_2 + NH_3 + 4\,OH^-$$

In strongly alkaline solution, where the wave-height corresponds to the consumption of two electrons per molecule, a two-electron reduction was found accordingly. As the reduction product gave an anodic wave as that given by hydrazides and hydrazines and as no volatile bases were detected in the reaction mixture, the product was assumed to be a dihydroisonicotinic hydrazide.

The reaction thus is:

$$NC_5H_4CO-N^--NH_2 + 2\,e^- + 2\,H_2O \rightarrow HNC_5H_5CO-N^--NH_2 + 2\,OH^-$$

In mineral acid solution 1,2-diisonicotinoyl hydrazine can be reduced in a reaction which consumes 6 electrons per molecule, and pyridine-4-aldehyde can be isolated in good yield. If allowed to proceed to completion at the potential used for the reduction of the compund, the aldehyde thus formed is reduced as mentioned in the reduction of isonicotinic amide. The over-all electron consumption is then 10 electrons per molecule. The reduction to aldehyde is:

$$HN^+C_5H_4CONHNHCOC_5H_4N^+H + 6\,e^- + 8\,H^+ \rightarrow 2\,NH_4^+ + \left\{\begin{array}{l} 2\,NH^+C_5H_4CHO + 2\,H_2O \\ \qquad \downarrow\!\uparrow \\ 2\,HN^+C_5H_4CH(OH)_2 \end{array}\right.$$

DISCUSSION

The anodic wave of isoniazid has the height of a four-electron wave at concentrations normally used in polarography. At higher concentrations the apparent electron consumption diminishes. In a phosphate buffer pH 11.2, $n = 2$ is found and in 0.2 M potassium hydroxide $n = 2.9$. These facts can be explained by the reaction scheme suggested above.

The four-electron oxidation found at low concentrations changes to a two-electron reaction in a phosphate buffer at higher concentrations of isoniazid. According to the scheme the reason is, that in the latter case the isoniazid-anion competes more successfully with the hydroxyl ions in the reaction with the isonicotinoyl-cation than in the former. The product, 1,2-diisonicotinoyl-hydrazine, can not be oxidized at the anode, so the over-all electron consumption becomes two electrons per molecule instead of four.

In more alkaline solution the isoniazid-anion is less favored in the competition with hydroxyl ions for the isonicotinoyl-cation, and more isonicotinic acid is formed. The electron consumption is thus between 2 and 4. Under normal polarographic conditions the product is assumed to be isonicotinic acid with no formation of 1,2-diisonicotinoyl-hydrazine. This reaction requires 4 electrons per molecule.

In order to explain the presence of pyridine-4-aldehyde in the oxidation mixture one can assume that some of the primarily formed $NC_5H_4CO-N=N^-$ diffuses away from the electrode before it looses its nitrogen. Some of the pyridine-4-aldehyde formed in this way is probably oxidized to isonicotinic acid at the anode. Pyridine-4-aldehyde was found to give an anodic wave in alkaline solution, and this wave proved to be caused by an oxidation to isonicotinic acid.

No sudden change in the $E_{\frac{1}{2}}$-pH-curve or other polarographic property has been found for the anodic wave between pH 8 and 13.5. The same species seems thus to be oxidized between pH 8 and 13.5. At pH 13.5 the isoniazid exists as the anion, and it is, therefore, assumed, that the isoniazid-anion is responsible for the occurrence of the anodic wave.

The pK for the dissociation of isoniazid is 10.77 [3]. The anodic wave of isoniazid is found at pH as low as 8. About pH 8 the isoniazid exists mainly as the undissociated molecule in the bulk of the solution. If the above mentioned assumption on the nature of the oxidizable species is correct, a dissociation has to take place before the oxidation. At the surface of the anode the isoniazid-anion is removed from the dissociation equilibrium by oxidation as fast as it is formed. The wave-height would thus be expected to be governed by the rate of the dissociation at low pH-values.

From the dependence of the wave-height on the height of the mercury reservoir can be concluded, that the wave-height is not kinetically controlled at pH 8.3 at a concentration about 5×10^{-4} M. At lower pH the anodic wave merges with the wave caused by the anodic dissolution of mercury.

At a concentration of 10^{-2} M isoniazid in a 0.5 M borate buffer pH 9 the height of the anodic wave changes somewhat with the height of the mercury reservoir, but not as much as required by a diffusion controlled wave. The height of the cathodic wave is diffusion controlled, as it is proportional to the square root of the height of the mercury reservoir. This shows, that the deviation from proportionality to the square root of the height of the mercury reservoir shown by the anodic wave is not caused by a too low buffer concentration, but rather by a partly control of the wave-height by the rate of the dissociation.

In a preparative oxidation of isoniazid at pH 11.2 a current density of 2 mA/cm^2 could be used, but at pH 13 10 mA/cm^2 or more could be obtained at the desired anode potential. This shows that in the former case the limiting current is not governed by the diffusion of isoniazid to the anode.

The finding the 1,2-diisonicotinoyl-hydrazine and isonicotinic acid are the main products from the oxidation of isoniazid in alkaline solution is not surprising in view of the fact that the oxidation of isoniazid with mercuric oxide yields these compounds. Also in the autoxidation of isoniazid [4-6] 1,2-diisonicotinoyl-hydrazine is formed together with isonicotinic acid.

Whether the electrons in the oxidation of isoniazid are transferred to the anode directly or *via* mercuric ions is not clear. Mercuric ions form a precipitate with isoniazid, but no such precipitate has been detected during the preparative oxidation of isoniazid at a mercury anode. A formation of such a precipitate with mercuric ions is found for many compounds giving an anodic wave. It is, however, not a sufficient condition for the formation of an anodic wave as, *e.g.*, 1-isonicotinoyl-2,2-dimethylhydrazine gives a precipitate with mercuric ions, but yields no anodic wave.

In the oxidation of 1-isonicotinoyl-2-phenyl-hydrazine an intermediate as that proposed above is likely. It is made plausible by the fact that during the beginning of the oxidation in 0.2 M potassium hydroxide a wave with $E_{\frac{1}{2}} = -0.8$ V *vs* S. C. E. grows up and disappears gradually during the later part of the oxidation. In the further oxidation of this intermediate mercuric ions seem to play a role. The reaction path is not clear, and a study of the anodic reaction of hydrazines and hydrazides is in progress.

The first cathodic wave of isoniazid is caused by a reductive splitting of the nitrogen-nitrogen bond. In strongly acid solution the half-wave potentials of this wave are independent of pH. The change in slope of $E_{\frac{1}{2}}$ *vs* pH occurs around pH 1.5. It is noteworthy that the p*K* for the equilibrium $HN^+C_5H_4-CONH-NH_3^+ \rightleftharpoons H^+ + HN^+C_5H_4CONH-NH_2$ is 1.85[3]. The corresponding p*K* for 1-isonicotinoyl-2-phenyl-hydrazine must be lower due to the influence of the phenyl group. Accordingly, no region is found, in which $E_{\frac{1}{2}}$ of the first wave of 1-isonicotinoyl-2-phenyl-hydrazine or 1-isonicotinoyl-2-methyl-2-phenyl-hydrazine is independent of pH.

The reductive splitting of the nitrogen-nitrogen bond in the reduction of isoniazid resembles the reduction of phenylhydrazones in which the first step[7] is assumed to result in a cleavage of the phenylhydrazone to an azomethine compound and aniline. The azomethine compound thus formed is, in contrast to the isonicotinic amide formed in the reduction of isoniazid, reducible at the potential necessary for the reductive splitting of the nitrogen-nitrogen bond. The azomethine compound is, therefore, reduced immediately, so the over-all reaction becomes a four-electron reduction.

A similar reduction of a hydrazide to an amide is found in the above mentioned derivatives of isonicotinic hydrazide, 1-ethyl-4-carbohydrazide-pyridinium bromide, thiazole-2-hydrazide, picolinic hydrazide and nicotinic hydrazide. In the latter case two waves are observed only between pH 0 and 4; at lower and higher pH-values the waves merge.

The reduction of isonicotinic amide, which causes the second cathodic wave of isoniazid, will be discussed in a later communication together with a discussion of the reduction of substituted isonicotinic amides.

Between pH 9 and 11 the four-electron reduction results in the formation of a dihydroisonicotinic amide and ammonia. The half-wave potentials of isoniazid and isonicotinic amide are in this pH-region nearly equal. The first step in the reaction is probably a reductive splitting of the nitrogen-nitrogen bond as found at lower pH-values. The isonicotinic amide thus formed is in alkaline solution reducible at the potential necessary for the reduction of the nitrogen-nitrogen bond.

In strongly alkaline solution the reduction consumes two electrons per molecule and the product is a dihydroisonicotinic hydrazide. The formation of the isoniazid-anion causes the nitrogen-nitrogen bond to be less easily reduced, and the compound then behaves as most other derivatives of isonicotinic acid, where the reduction in alkaline solution takes place in the pyridine nucleus.

EXPERIMENTAL

The polarograph was a recording polarograph Radiometer PO 4d. The potentiostat was a Wadsworth Controlled Potential Electro-Depositor. The capillary delivered 2.45 mg of mercury per second at a corrected mercury column height of 48.5 cm. The drop time was 3.7 sec. (H_2O, open circuit).

Materials. Isoniazid (Fluka). 1-Isonicotinoyl-2-methyl-2-phenyl-hydrazine, m.p. 143.5°—144°, was a gift from Dr. F. Lund, Leo Pharmaceutical Products. It was prepared by reaction between the mixed anhydride of benzoic and isonicotinic acid and 1-methyl-1-phenyl-hydrazine. In a similar way 1-isonicotinoyl-2-phenyl-hydrazine, m.p. 190°—191° (185°—186° [8]), was prepared. 1-Isonicotinoyl-2,2-dimethyl-hydrazine, m.p. 120°—121°, was prepared according to Fox and Gibas [9].

Oxidation of isoniazid in a phosphate buffer. Isoniazid (0.500 g) was dissolved in 170 ml of a deaerated 0.3 M phosphate buffer pH 11.2 containing 1 M potassium chloride. The temperature was kept between 0° and 5°. The stirred solution was oxidized at an anode potential of 0.00 V *vs* S.C.E. The highest current density which could be used under these conditions was 2 mA/cm^2. The oxidation required 2 electrons per molecule. The reaction was followed polarographically, and after the oxidation was completed 1,2-(diisonicotinoyl)-hydrazine and pyridine-4-aldehyde were found in 90 % and 5 % yield, respectively. The presence of an aldehyde in the reaction mixture was also shown qualitatively by the formation of a 2,4-dinitrophenylhydrazone. The reaction mixture was extracted 6 times with 40 ml butanol, and the combined butanol extracts was extracted with 20 ml N HCl. On neutralization of the acid with solid sodium carbonate a precipitate, 357 mg (80 %), was obtained, which was identified as 1,2-(diisonicotinoyl)-hydrazine from its m.p. 255°—257° (258°—259°) [5] and IR-spectrum.

In a similar way isoniazid was oxidized at 0.0 V *vs* S.C.E. in 0.2 M potassium hydroxide containing 1 M potassium chloride. The current density could under these conditions be more than 10 mA/cm^2. The oxidation required 2.9 electrons per molecule, and isonicotinic acid (45 %) and 1,2-(diisonicotinoyl)-hydrazine (55 %) were determined polarographically after the completion of the oxidation. No other products were detected.

1-Isonicotinoyl-2-phenyl-hydrazine was oxidized similarly in a phosphate buffer pH 11.9. The oxidation required 2.5 electrons per molecule. Isonicotinic acid was formed in more than 90 % yield (determined polarographically). Some gas, probably nitrogen, was evolved during the oxidation. A grey precipitate was formed, which on extraction with pyridine or carbon tetrachloride yielded mercury and a yellow solution. The IR-spectrum of this solution gave no evidence for the presence of benzene, phenylazide, biphenyl, azobenzene, or diphenyl mercury. Traces of aniline could be detected by diazotization and coupling.

Reduction of isoniazid. Isoniazid (0.500 g) was reduced in 0.4 N HCl at a cathode potential of 0.50 V *vs* S.C.E. The cathode potential was kept near the half-wave potential of the first wave; a potential on the plateau of the first wave could not be used, as the difference between the reduction potentials of the first and second wave was too small to permit this. After completion of the reduction which consumed two electrons per molecule the solution was made slightly alkaline with solid sodium carbonate and extracted twice with 1-butanol. The butanol was washed with a few ml of water and evaporated *in vacuo*. The residue, 265 mg (74 % yield), was identified as isonicotinic amide through the m.p. 156° (Litt. 155°—156°) and IR-spectrum.

In a similar way *1-isonicotinoyl-2-phenyl-hydrazine* was reduced at the half-wave potential of the first wave. The separation of the two waves is not as good at high as at low concentrations. The isonicotinic amide formed in the reduction was determined polarographically, and the aniline was determined colorimetrically after diazotization and coupling.

Isoniazid was reduced in a phosphate buffer pH 11. The reduction consumed four electrons per molecule. Ammonia was formed during the reduction, but the structure of the dihydroisonicotinic amide was not established.

In a 0.2 M potassium hydroxide solution the reduction of isoniazid consumed two electrons per molecule. No volatile bases were detected after the reduction. The cathodic wave disappeared gradually from the polarograms of the reaction mixture, but an anodic wave was still found in the fully reduced solution. — The structure of the dihydroisonicotinic hydrazide was not determined.

1-Isonicotinoyl-2-phenyl-hydrazine was reduced in 0.2 M potassium hydroxide solution. The reduction consumed four electrons per molecule. Aniline was formed in more than 90 % yield, which was determined colorimetrically after diazotization and coupling.

REFERENCES

1. Anastasi, A., Mecarelli, E. and Novacic, L. *Microchemie ver. Mikrochim. Acta* **40** (1952) 113.
2. Maruyama, M. *J. Pharm. Soc. Japan* **72** (1952) 1213.
3. Albert, A. *Experientia* **9** (1953) 370.
4. Albert, A. and Rees, C. W. *Biochem. J.* **61** (1955) 128.
5. Krüger-Thiemer, E. *Naturwiss.* **42** (1955) 47.
6. Winder, F. G. and Denneny, J. M. *Biochem. J.* **73** (1959) 500.
7. Lund, H. *Acta Chem. Scand.* **13** (1959) 249.
8. Yale, H. L., Losse, K., Martins, J., Holsing, M., Perry, F. M. and Bernstein, J. *J. Am. Chem. Soc.* **75** (1953) 1933.
9. Fox, H. H. and Gibas, J. T. *J. Org. Chem.* **20** (1955) 60.

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