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Oxidation-Reduction Potentials of Certain Synthetic Flavins.

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The electrochemical properties of natural and synthetic lactoflavin and their relation to chemical structure as well as to biological activity have been well established. Furthermore, the oxidation-reduction potentials, E'_o, of a large series of substituted flavins have been determined by Kuhn and Boulanger.[1] They found that the index potentials, E_i, are greater than 14 millivolts which would correspond to 2 electron transfer, and less than 28 millivolts which would correspond to one electron transfer. This suggested a semiquinone-formation. Michaelis and coworkers[2] demonstrated the presence of a colored semiquinone over the entire pH range studied, and determined its formation-constant, quantitatively. While the work of these investigators was in progress, the present authors were independently studying the oxidation-reduction potentials of synthetic lactoflavin and 2 substituted flavins prepared essentially according to the methods described by Karrer.[3] The results of our experiments are briefly summarized in Table I, in which E'_o is the normal potential at the stated pH, and E_i designates the index potential.

Although data on our substituted flavins, (7-mono-methyl-d-arabinose-flavin and 7-mono-methyl-l-arabinose-flavin), have not been previously reported, they are to be regarded merely as confirming Kuhn and Boulanger's findings that the potentials are largely independent of the sugar attached to the alloxazin ring and that they are only slightly shifted to the positive side if one of the 2 methyl groups is removed. Our data on the potential of lactoflavin are in essential agreement with those previously given for natural and synthetic lactoflavin.[1, 2, 4, 5] In agreement with the results of Michaelis, Schubert and Smythe,[2] we also found that the titration curve of the lactoflavin is less steep than would be expected on the basis of a

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[1] Kuhn, R., and Boulanger, P., *Ber. chem. Ges.*, 1936, **69**, 1557.

[2] Michaelis, L., Schubert, M. P., Smythe, C. V., *J. Biol. Chem.*, 1936, **116**, 587.

[3] Karrer, P., Becker, B., Benz, F., Frei, P., Salomon, H., Schöpp, K., *Helv. Chim. Act.*, 1935, **18**, 1435.

[4] Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem.*, *(Proc.)*, 1934, **105**, vii.

[5] Stare, F. S., *J. Biol. Chem.*, 1935, **112**, 223.

TABLE I.

6,7-Dimethyl-d-ribose-flavin (Lactoflavin).

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
1.13	n/10 HCl	+122	19	(R) $TiCl_3$	
4.52	Acetate	— 86	18	(R) "	
4.52	"	— 88	17	(O) Benzo quinone	++
6.94	Phosphate	—207	16	(R) $Na_2S_2O_4$	
6.94	"	—207	16	(O) Naphthol-sulfonate-indophenol	+
6.98	"	—205	18	(R) $Na_2S_2O_4$	
6.98	"	—205	18	(O) Dichlorophenol-indophenol	+
7.68	"	—223	17	(R) $Na_2S_2O_4$	
7.68	"	—223	18	(O) Dichlorophenol-indophenol	+
10.52	"	—343	18	(R) $Na_2S_2O_4$	
10.52	"	—343	18	(O) Naphthol-sulfonate-indophenol	+
10.99	"	—365	18	(R) $Na_2S_2O_4$	
10.99	"	—365	18	(O) Naphthol-sulfonate-indophenol	+
11.41	"	—399	17	(R) $Na_2S_2O_4$	

7-Mono-methyl-d-arabinose-flavin

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
1.13	n/10 HCl	+132	17	(R) $TiCl_3$	
4.52	Acetate	— 74	16	(R) "	
5.61	Phosphate	—141	14	(O) Dichlorophenol-indophenol	++
6.18	"	—164	15	(O) " "	++
6.91	"	—192	16	(O) " "	++
7.70	"	—216	16	(O) " "	++
11.41	"	—376	16	(O) Naphthol-sulfonate-indophenol	++

7-Mono-methyl-l-arabinose-flavin

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
4.52	Acetate	— 77	16	(R) $TiCl_3$	
6.41	Phosphate	—174	16	(O) Naphthol-sulfonate-indophenol	++
7.71	"	—213	16	(O) " " "	++
10.59	"	—324	17	(O) " " "	++

\+ Solution reduced by preceding titration.
++ Solution previously reduced by H_2 and colloidal palladium.

2-electron transfer, over the entire range of pH studied, suggesting a semiquinone formation. The potentials of 7-methyl-d-arabinose-flavin are only slightly more positive than those of lactoflavin, the index potential being less than that of lactoflavin. The potentials of 7-methyl-d-arabinose-flavin are essentially identical with those of the l-arabinose compound. The dissociation constants of the substances titrated are practically the same as indicated by the curves of E'_0 against pH.

Due to the fact that the amounts of material available were quite limited, a vessel was devised which permitted the titration of 0.6 mg. of flavin dissolved in 10 cc. of buffer. In some cases, the curve of oxidation was determined on the same solution as the curve of reduction. Good agreement was found between the two curves. The oxidants and reductants used are indicated in the table.