Isoalloxazine (Flavin) Dehydrogenation of Dimethyl *trans*-1,2-Dihydrophthalate

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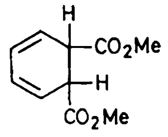
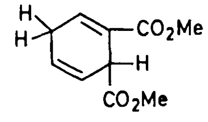
Summary The reaction of dimethyl *trans*-1,2-dihydrophthalate (I) and dimethyl 1,4-dihydrophthalate (II) with a series of flavins involves reaction of the common dimethyl dihydrophthalate carbanion and flavin(s) within a face-to face complex not unlike that formed between flavin and tryptophan; the 10a-position is not involved in this reaction and is not likely involved in the reoxidation of the flavins by O_2.

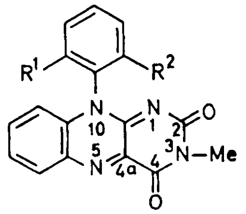
DIMETHYL *trans*-1,2-DIHYDROPHTHALATE (I) under anaerobic conditions (λ_{max} 255 nm) isomerizes to dimethyl 1,4-dihydrophthalate (II) *via* specific base catalysis (30°; H_2O; k_{OH} 233 M^{-1} min^{-1}). The assignment of structure (II) is based on n.m.r. analysis [δ (CCl_4): 2·85—3·2 (2H, m), 3·69 (3H, s), 3·76 (4H, s), 5·8—5·9 (2H, m), and 6·98—7·10 (1H, m) p.p.m.] and the disappearance of the absorbance at 255 nm (λ_{max} for a cyclic conjugated diene). 1,2-Dihydrophthalic acid (III) has been reported to rearrange in boiling water to 4,5-dihydrophthalic acid.[1] However, the rearrangement product from the dicarboxylic acid when



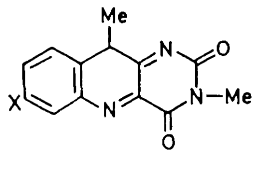
(I)



(II)



(IV) $R^1 = R^2 = Me$
(V) $R^1 = H$; $R^2 = Me$
(VI) $R^1 = R^2 = H$



(VII) X = Cl
(VIII) X = H

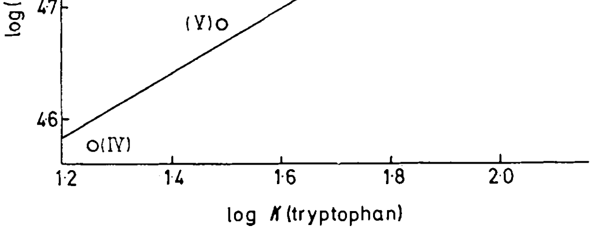


FIGURE. *log* (k_b/k_{-a}) vs. *log* K_1 *for equilibrium complexing of the isoalloxazines with tryptophan. The slope* (0·29) *is less than that recorded for NADH* [2·25 *for* (IV)—(VIII)]. *The inset shows log* (k_b/k_{-a}) vs. *log* k_{2nd}^{NADH} (*slope* 0·14).

treated with diazomethane yields a product indistinguishable from (II).

Reactions of (I) with the flavins (IV)—(VII) where $[(\mathrm{I})] \gg [\text{flavin}]$, followed the kinetic scheme (1). The dependence

$$\mathrm{CH} \underset{k_{-a}}{\overset{k_a}{\rightleftarrows}} \mathrm{C}^- \xrightarrow{k_b} \text{Dimethylphthalic acid} \qquad (1)$$

$$k_a = k_{\mathrm{HO}}[\mathrm{HO}^-], \quad k_{-a} = k_{\mathrm{H_2O}}[\mathrm{H_2O}], \quad k_b = k_f[(\mathrm{IV})\text{—}(\mathrm{VIII})]$$

of the initial rate upon pH (range of pH 9·8—11·3) was determined. From these results values of the rate of reaction of (I) with (VI)—(VIII) were found to be invariant and to follow the rate law (2). Values of k_b/k_{-a} were

$$\mathrm{d}[\text{Flavin}]/\mathrm{d}t = 233\ [(\mathrm{I})]\ [(\mathrm{IV})\text{—}(\mathrm{VIII})] \qquad (2)$$

determined *via* analogue computer fitting of (2) to the experimental data. The Figure shows plots of $\log(k_b/k_{-a})$ for (I) *vs.* the equilibrium constants (K_1) for the 1 : 1 complexing of tryptophan by flavins (IV)—(VIII) determined *via* fluorescence quenching. The positive linear relationship suggests that the carbanion of (I) forms a complex with flavin prior to reduction of flavin and aromatization of (I).

We have previously noted[2] that log k_{rate} for reaction of NADH and n-propylnicotinamide with flavins is a linear function of log K_1 (see insert to Figure) but that the rates of addition of $\mathrm{SO_3}^{2-}$ to and oxidation of the monoanion of butane-1,4-dithiol by (IV)—(VIII) are linear functions of the reduction potential of (IV)—(VIII). This suggests two classes of (dark) reactions of flavins: (i) class C, in which the rate of reaction is dependent on the face-to-face complexing affinities of flavin and reagent, and (ii) class N, in which the rate is dependent upon the affinity of the isoalloxazine for electrons (charge transfer complexing for class C and covalent addition[3] for class N ?).

The 10a- and 4a-positions have been considered as possible sites for initiation of reactions of isoalloxazines *via* covalent addition of nucleophilic species[3] and also as the possible positions for attack of $\mathrm{O_2}$ upon reduced flavins.[4] The steric hindrance to approach of the 10a-position by the 2,6-dimethyl substituents of the benzene ring of (IV) eliminates this position as a seat of reaction for all the compounds investigated by us [log (k_b/k_{-a}) for (IV) does not deviate from the plot in the Figure]. The ready reoxidation of reduced (IV) by $\mathrm{O_2}$ (stopped-flow time period) strongly suggests that the 10a-position may be ignored for this reaction also.

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