

ACTIVATION AND TRANSFER OF OXYGEN VIII. AUTOXIDATIVE RINGCONTRACTION OF BLOCKED DIHYDROALLOXAZINES AND TETRAHYDROPTERIDINES IN THE PRESENCE OF  $H_2^{18}O$  AND  $^{18}O_2$ .

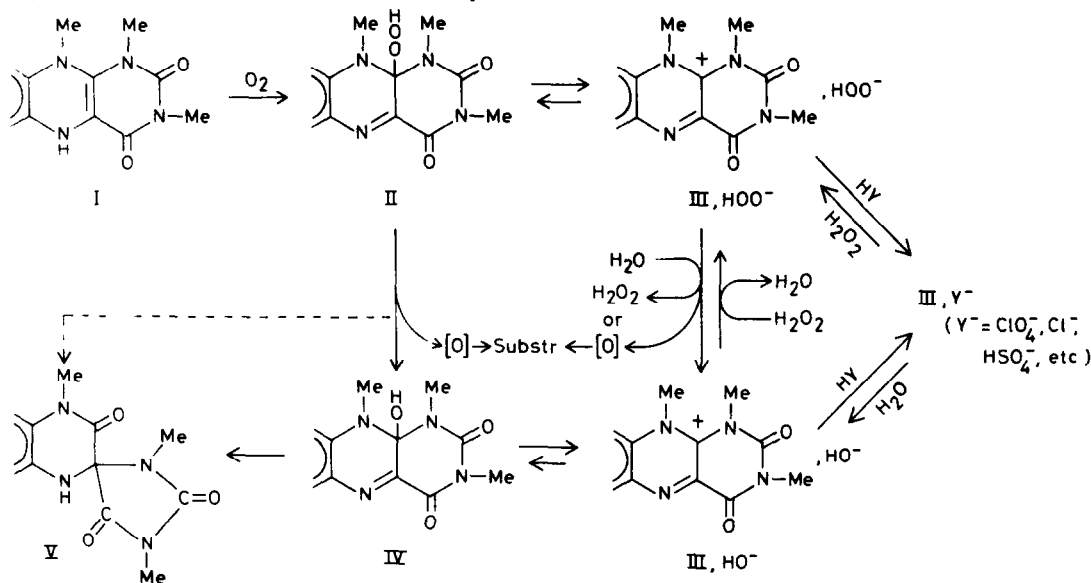
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Oxygen transfer like non-enzymic aromatic hydroxylations could be effected by systems containing a dihydroflavin or a tetrahydropteridine. The results described<sup>1-5</sup> are consistent with the occurrence of transient hydroperoxides like (II) which could also be considered as a pseudo-salt (III,  $HOO^-$ ).

$H_2O_2$  production and oxygen transfer are accompanied by the formation of a carbinolamine/pseudobase (IV  $\rightleftharpoons$  III,  $HO^-$ ). These species are rapidly converted into either: 1) the spirohydantoin (V) in anhydrous solutions or in aqueous media of  $pH > 2$ , or 2) quaternary salts (III,  $Y^-$ ) in more acid solution.



Additional evidence for the above scheme has now been obtained from  $^{18}O$  labelling studies. The spirohydantoin ( $m/e=274$ ) was prepared under various conditions from 0.1 mmole of (III,  $Y^-$ ) or (I) per 2 ml of solvent at  $23^\circ$ :

1) neutralization of III,  $Y^-$  in  $H_2^{16}O$  afforded V according to III,  $Y^- \rightarrow$  III,  $HO^- \rightarrow$  IV  $\rightarrow$  V. The M, M+1 and M+2 peaks were used to correct the mass spectra of the products from the

following experiments taken under the same conditions; 2) neutralization of III,  $Y^-$  in  $H_2^{18}O$  (13% enrichment) gave a mass spectrum with a corrected intensity ratio of  $M/M+2=1:0.15$ , corresponding with 13% enriched spirohydantoin; 3) autoxidation of I +  $^{16}O_2$  in  $H_2^{18}O$  gave a lower enrichment, indicating that about 10% of V contained  $^{16}O$  from the gas-phase. Because of the relatively low concentration of  $^{18}O$  in the water, further evidence had to be obtained from autoxidations by  $^{18}O_2$  (93.5% enrichment). The following manometric experiments were performed: 4) I +  $^{16}O_2$  in  $H_2^{16}O$  as the blank; 5) I +  $^{18}O_2$  in 1N acetic acid/ $H_2^{16}O$  (pH=2.5); 6) I +  $^{18}O_2$  in 0.1 M borax/ $H_2^{16}O$  (pH=9.2); 7) I +  $^{18}O_2$  in dried solvents e.g. benzene.

The  $^{18}O$  enrichment of the spirohydantoin obtained from Expt. 5 and 6 was 3-4% and 13%, respectively. Recrystallization of labelled V from boiling 1N AcOH/ $H_2^{16}O$  did not lead to loss of label. Therefore, the results given by Expts. 5 and 6 demonstrate the  $^{18}O$  -  $^{16}O$  exchange in aqueous solution through the carbinolamine/pseudobase equilibrium ( $IV \rightleftharpoons III, HO^-$ ), influenced by the pH. Consequently, autoxidation in the presence of  $^{18}O_2$  in anhydrous solutions (Expt. 7) led to abundantly labelled spirohydantoin (80% of the expected label).

Similar experiments were performed with 1,3,6,7,8-pentamethyl-5,6,7,8-tetrahydropteri-2,4-dione, giving an autoxidative ringcontraction into a piperazine-spirohydantoin<sup>5</sup>. For example, autoxidation of 0.5 mmole of  $H_4Pter + ^{18}O_2$  in 10 ml of non-buffered  $H_2^{16}O$  resulted into 2-3% enriched spirohydantoin. On the other hand, a product with  $M/M+2=1.6$  was obtained by Blair and Pearson<sup>6</sup> in the experiment:  $H_4Pter + ^{16}O_2 + H_2^{18}O$  (43.46% enrichment) in 90% pyridine. From this it could be calculated that 12-13% of the spirohydantoin then contained  $^{16}O$  from the gasphase. However, these authors did not arrive at this right conclusion. They miscalculated the theoretical  $M/M+2$  ratio on 1.53, while it should be 1.28, if the natural  $M+2$  peak is also taken into consideration. Blair and Pearson did not use  $^{18}O_2$ . They neither studied the labelling at various pH nor the  $^{18}O$  incorporation in non-aqueous media.

Conclusion: Our labelling experiments do not provide a basis for the alternative mechanism proposed by Blair and Pearson.

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