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MECHANISM OF QUERCETIN OXYGENATION
A POSSIBLE MODEL FOR HAEM DEGRADATION

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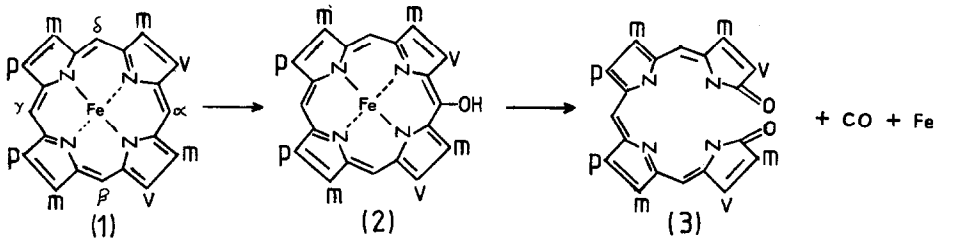
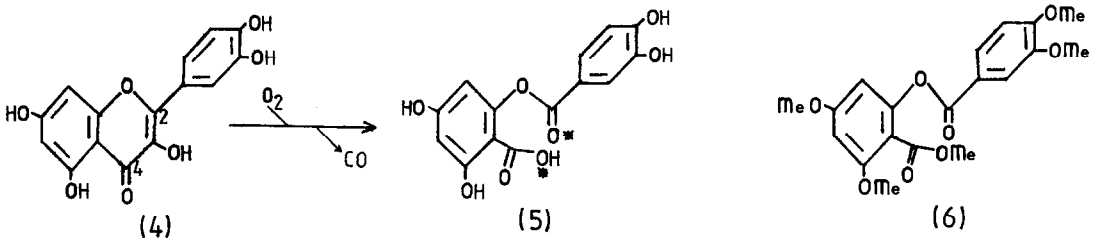
Summary: Quercetin undergoes base-catalysed oxidative decarbonylation by a mechanism involving only one oxygen molecule, unlike the apparently similar step in haem catabolism, which requires two oxygen molecules.

The degradation of haem (1) to the bile pigments biliverdin (3) and bilirubin represents the major physiological pathway for the disposal of the haem of haemoglobin and other haemoproteins[1,2]. An early intermediate in this degradation is thought to be mesohydroxyhaem (2), which then reacts further with molecular oxygen yielding biliverdin, iron and CO[2]. ^{18}O labelling work, both *in vivo* and *in vitro* has demonstrated that the lactam oxygen atoms incorporated into bile pigments during formation from haem are derived from two different oxygen molecules (Two-Molecule Mechanism) and not from the same oxygen molecule (One-Molecule Mechanism)[3-6]. The molecular pathway responsible for this reaction has not yet been satisfactorily elucidated, in spite of its importance in metabolism. No analogous reactions occurring by a Two-Molecule Mechanism have yet been reported, but appropriate chemical models might be very useful in determining the molecular mechanism of haem degradation.

Quercetin (4), a flavonol, is present in the leaves and flowers of higher plants as the 3-O-glycoside, rutin, which contributes a cream pigmentation. The first step in the degradation of quercetin in living systems is oxidative decarbonylation to depside (5). This step involves the insertion of two oxygen atoms (asterisked in (5)), at C-2 and C-4 and elimination of C-3 as carbon monoxide, a reaction which is formally analagous to the ring cleavage step in haem degradation. Quercetin oxygenation can be performed *in vitro* by the dioxygenase quercetinase[7] and also non-enzymically by base-catalysed (^tBuOK) oxygenation[8], photosensitised oxygenation[9] and metal-ion (Co^{III} or Cu^{II}) catalysed oxygenation[10].



Abbreviations: M, $-CH_3$; V, $-CH=CH$; P, $-CH_2CH_2CO_2H$



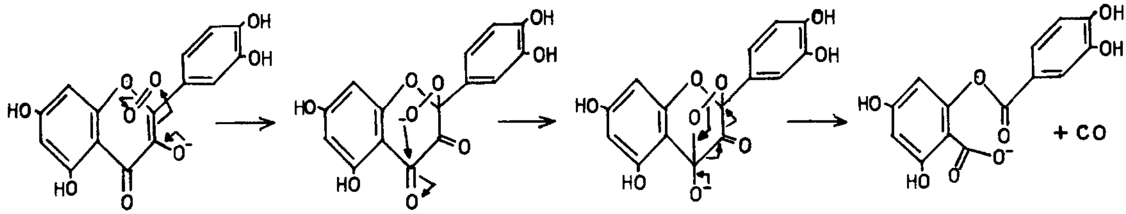
By the use of $^{18}O_2$ and $H_2{}^{18}O$ labelling, it has been shown that the oxygen atoms incorporated during the quercetinase-catalysed oxygenation are derived from molecular oxygen[7]. This would also be expected in the non-enzymic reaction systems[8-10], since these reactions were carried out in non-aqueous media (DMF or DMSO). However, no experiments have hitherto been carried out to determine whether quercetin oxidation occurs by a One-Molecule Mechanism or a Two-Molecule Mechanism. In view of the apparent analogy of the reaction to haem degradation, we now report such experiments using ^{18}O labelling.

The method depends upon the use of ^{18}O enriched oxygen containing $^{18,18}O_2$ and $^{16,16}O_2$, but none of the mixed species $^{18,16}O_2$, followed by mass spectral analysis of the reaction product. The One-Molecule Mechanism predicts incorporation of label at m/e M+4 but not at m/e M+2 (where M is the mass of the molecular ion of the product or an appropriate derivative), since the reacting oxygen molecules contain either both atoms labelled or both atoms unlabelled. However the Two-Molecule Mechanism predicts incorporation both at m/e M+2 and at m/e M+4. The predicted incorporations can readily be expressed on a quantitative basis[3], (see Table 1).

A closed reaction vessel, containing unmixed solutions of quercetin (70 mg) in DMF (0.1 ml) and ^{t}BuOK (35 mg) in DMF (2 ml), was degassed and an appropriate mixture of $^{18,18}O_2$-$^{16,16}O_2$ was introduced. A small sample of the gas phase was removed before and after each reaction for

Table 1: ^{18}O Incorporation into the tetramethylether methyl ester of the depside (6) from base-catalysed oxygenation of Quercetin under 48% $^{18}O_2$.

		m/e	M	M+2	M+4
PREDICTED	(Two-Molecule Mechanism)		27.0	50.0	23.0
PREDICTED	(One-Molecule Mechanism)		52.0	0	48.0
OBSERVED	Experiment 1		52.6	0	47.4
OBSERVED	Experiment 2		57.8	0	42.2



SCHEME 1

analysis by mass spectrometry to determine the percent ^{18}O. After mixing of the reactants and incubation at 37°C for 3h, the reaction was stopped by cooling to 0°C. Following extraction in ether and purification by paper chromatography, the depside was methylated to the tetramethylether methylester (6) with diazomethane and isolated by t.l.c.

The mass spectrum of material isolated from reaction under 100% $^{18,18}O_2$ showed a large peak at m/e 380 (corresponding to m/e M+4), an almost insignificant peak at m/e 376 and no peak at m/e 378 (corresponding to m/e M+2). This result confirms that the newly incorporated oxygen atoms are derived from molecular oxygen and also demonstrates the absence of any significant exchange of labelled oxygen with the medium during the work-up.

The spectra of material isolated from two experiments carried out under 48% $^{18,18}O_2$ showed large peaks at m/e 376 and m/e 380 but, after correction for naturally abundant isotopes, showed no incorporation at m/e 378. These observations immediately suggest oxygenation by a One-Molecule Mechanism (see above). The mass spectral data have been analysed quantitatively by the method described in detail elsewhere[3]. The results are shown in Table 1 from which it is clear that there is satisfactory quantitative correlation with the predictions for a One-Molecule Mechanism, in contrast to the results found for haem catabolism.

Our data are consistent with the mechanism suggested by Nishinaga *et al*[8] (Scheme 1). This involves formation of a ketohydroperoxy intermediate (7) by concerted addition of oxygen to the enol form of quercetin, followed by nucleophilic attack at C-4 to give a cyclic peroxide (8) which breaks down to the depside, releasing CO.

The present work establishes that non-enzymic base-catalysed oxygenation of quercetin occurs by a One-Molecule Mechanism. This fundamental mechanistic difference between haem and quercetin oxygenation, in spite of their stoichiometric similarity, excludes the use of quercetin as a chemical model in the study of haem catabolism. The present work further highlights the uniqueness of the haem degradation mechanism.

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