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The Mechanism of the Enzymic Induced Flavanone - Isoflavone Change.

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Abstract: A new scheme for the P 450 enzymic conversion of flavanones into isoflavones, which surmounts known experimental constraints, is proposed.

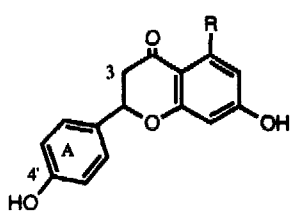
An important phase in the biosynthetic construction of rotenoids such as rotenone, amorphigenin and deguelin is the conversion of liquiritigenin (1) into 4'-O-methyldaidzein (6), which we showed involves migration of ring-A from C-2 of the former to C-3 of the latter.[1,2] This is a general problem of isoflavonoid biosynthesis which has been recognised for more than 30 years without a fully convincing mechanism being arrived at.[3] It has been shown that conversion of (1) into (4) or (2) into (5) is initiated by isoflavone synthase, a P-450 iron-containing microsomal enzyme of the endoplasmic reticulum of elicitor treated soybean.[4,5] The first product is (3) which is then dehydrated by a separate soluble enzyme: the latter has no requirement for NADPH or dioxygen.[4,5]

Any fully plausible mechanism must take account of certain constraints.

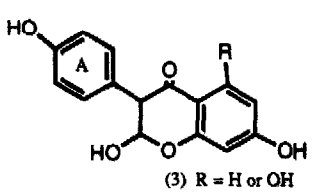
1. The reaction involves a cytochrome P-450 iron-containing enzyme which requires NADPH and dioxygen.[4,5]

2. A free p-hydroxyl is required in ring A. This emerges from our biosynthetic experiments[2,6] and from the fact that a p-methoxylated cinnamic acid destined for isoflavonoid biosynthesis must first be demethylated even though the final natural isoflavone is methylated in this position: it must be remethylated.[7-9]

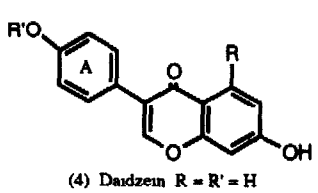
3. The emergent isoflavone usually has a free p-hydroxyl, but in rotenoid biosynthesis it is methylated and the methylation appears to be a part of the mechanism.[2,6]



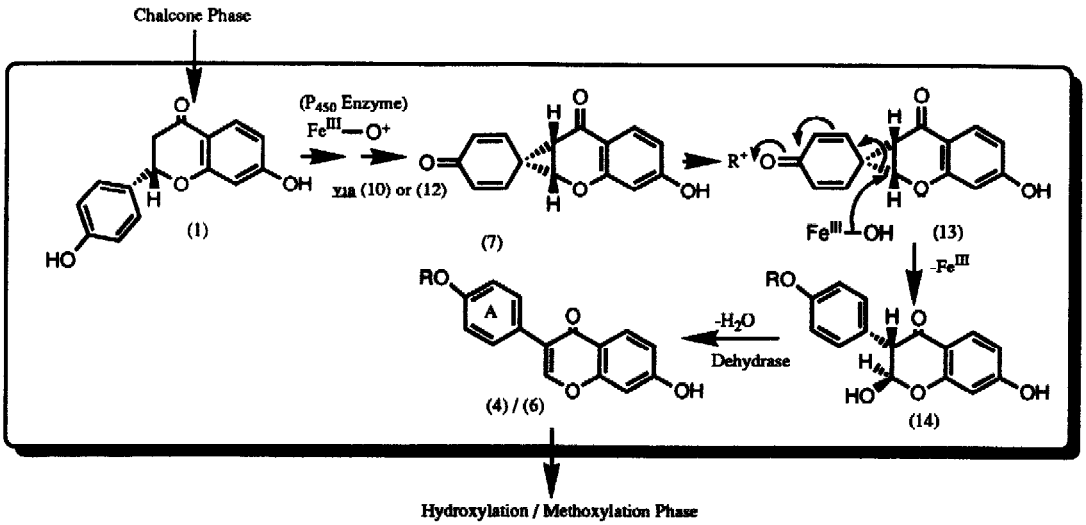
(1) Liquiritigenin R = H
(2) Naringenin R = OH



(3) R = H or OH



(4) Daidzein R = R' = H
(5) Genistein R = OH, R' = H
(6) Rotenoid intermediate R = H, R' =



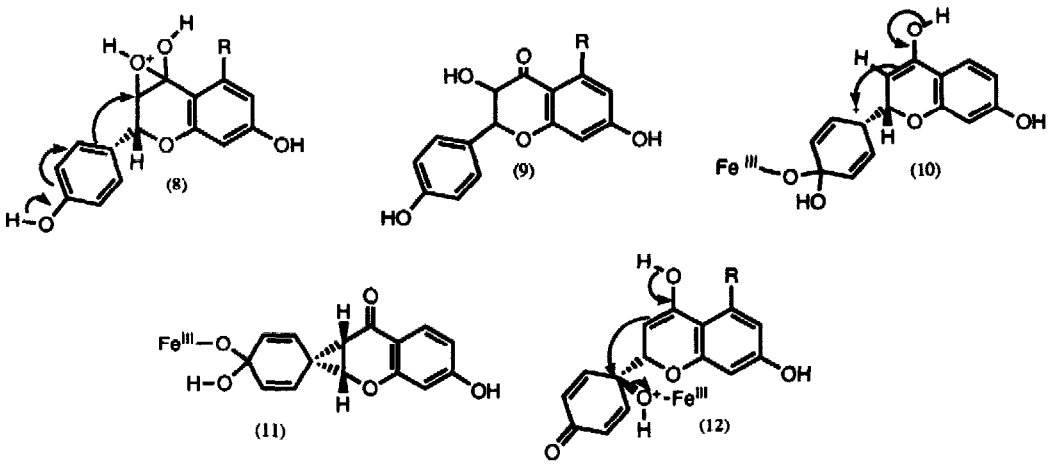
Scheme. The Flavanone / Isoflavone Phase: P_{450} Enzyme Mechanism.

4. Using microsomes from elicitor-treated Pueraria lobata, it has recently been shown that the oxygen of the 2-hydroxyl of (3) originates from dioxygen ($^{18}O_2$ experiment) and not water, but there is no evidence of dioxygen exchange elsewhere e.g at the 4'-hydroxyl.[10]

5. The processes involved should be chemically, biochemically, and enzymically acceptable.

On the basis of our earlier experimental evidence we have supported the spirodienone (7) as an acceptable intermediate in the rearrangement. This contrasts with the recent proposal of Sankawa and his colleagues in which it is proposed that the 3- hydrogen of the flavanone (1) or (2) is removed by the P-450 enzyme to give a radical which undergoes a 1,2-aryl shift, the new radical at C-2 being discharged by the enzyme in the $Fe^{iv}OH$ state.[10,11] No specific role is given to the para- hydroxyl of the aryl substituent and as judged by radical reactivities towards a series of substituted olefins, the postulated radical is moving from a more stabilised to a less stabilised site.[6] Grisebach et al.[4] accept the dienone (7) and suggest that the structure is attained by decomposition of a P-450 generated enol epoxide (8). The difficulty with this proposal is that one might expect the formation of a 3-hydroxyflavanone (9) rather than formation of a spiro-system.

Our proposal is that the origins of the dienone (7) lie in ipso substitution involving the P 450 enzyme in the electrophilic $Fe^{III}O^+$ resonance form.[12] Attack could be at C-1' or C-4'. In the latter case the process is akin to the initiation of ortho-hydroxylation, a process frequently represented as involving an arene epoxide.[13] Epoxidation is known not to be a concerted process,[12] and because of the structural situation the initial delocalised carbonium ion can be discharged by the enolate as shown (10) to give the spiro-compound (11) (it is known that the carbonyl in systems such as (1) is enolisable).[14] The latter eliminates $Fe^{III}OH$ to give the spirodienone (7)[2,15] and it will be observed that there is no exchange in an ^{18}O experiment with oxygen attached



to iron. An attractive alternative is that ipso-attack occurs at the nucleophilic C-1' position and is followed by formation of the dienone (7) by displacement.(12). Models show that the system is well disposed for this displacement and the $Fe^{III}OH$ is released at the enzyme active site close to C-2 atom with which it is to react in the next stage.

The dienone is now envisaged as being protonated at the carbonyl oxygen with the developing carbonium ion at C-2 (stabilised in the transition state by the adjacent lone pair of oxygen) attacked by the Fe^{III}–OH produced in the earlier stage ('rebound') (13, see Scheme). The product (14) is released from the enzyme leaving the active site in the heme Fe^{III} state ready to be reactivated after the binding of another molecule of substrate. In the case of rotenoid biosynthesis R^+ is thought not to be a proton but Me^+ derived from S-adenosylmethionine[2,6] thus daidzein (4) is not an acceptable precursor for the next stage of rotenoid biosynthesis, whereas (6) is.[2] The less attractive alternative would have to be a specific enzymic methylation which occurs on (14) but not (4).

The enzymic flavanone - isoflavone change occurs with (S)-(-)-liquiritigenin or -naringenin, but not the (R)- forms.[5,16,17] As indicated in the Scheme, this has certain consequences. Although no optical activity has been detected[5] the emergent hydroxy compound (14), being a β-ketoaldehyde hemiacetal, might be expected to have an easily racemised 2(R),3(S)-configuration, and what N.M.R. data are available,[10] indicate a probable mixture of forms..

Rotenoids of the Nyctaginaceae and Iridaceae do not retain a hydroxyl in ring-A which corresponds with the p-hydroxyl of liquiritigenin, but their biosynthesis is readily explained if the initiating acid is o-hydroxycinnamic acid , giving the 2'-o-hydroxy isomer of (1) in the Scheme.[6]

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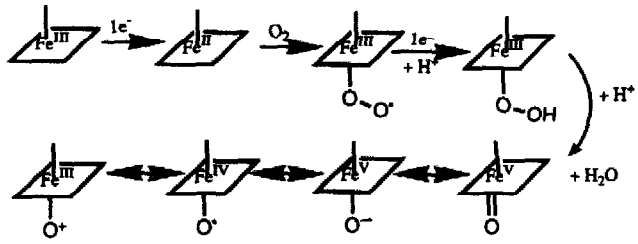
Activation of P 450 enzyme



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