Enzymic Oxidative Modification of Prenyl Groups. The Biosynthetic Origins of the *E*-Ring Systems of Rotenone and Amorphigenin

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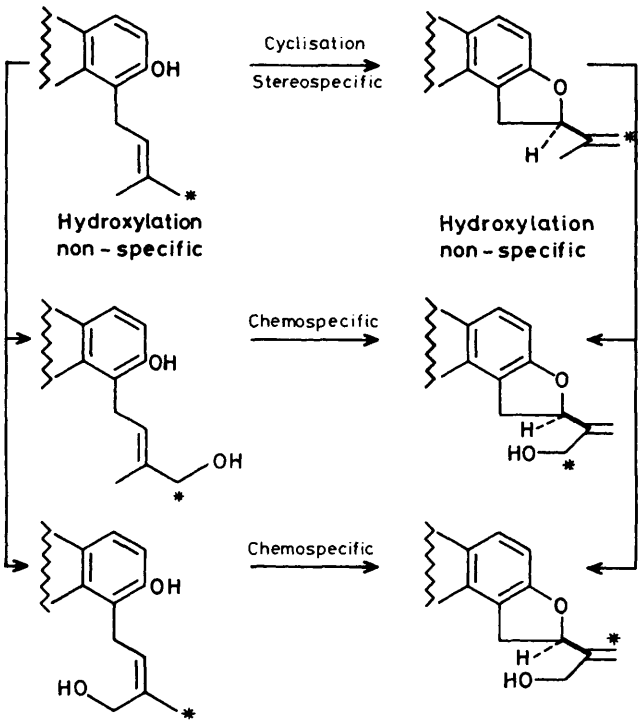
The major biosynthetic route to amorphigenin in *Amorpha fruticosa* is from rot-2'-enonic acid *via* cyclisation to rotenone with the (*E*)-methyl of the former becoming the methylene of the latter, followed by positionally non-specific hydroxylation; a subsidiary route involves positionally non-specific hydroxylation of rot-2'-enonic acid followed by chemospecific (for the methyl group) cyclisation to amorphigenin.

In our continuing study of rotenoid biosynthesis attention has lately been given to the ring-*E* appendages derived by oxidative modification of the hemi-terpenyl unit of rot-2'-enonic acid. During earlier work[1,2] we have shown that [4'-^{3}H]-rot-2'-enonic acid (**3**) is incorporated into amorphigenin (**2**) (0.77%) by *Amorpha fruticosa* seedlings, as is [7'-^{14}C]-rotenone (**1**) (0.57—1.89%) with approximately even scrambling of the carbon isotope between C-7' and C-8'. Because of the possibility of generating a symmetrical allyl species during work-up and degradation, three types of experimental work-up were employed, but the conclusion that the scrambling is part of the reaction mechanism remained unchanged.[1] Administration of [8'-^{14}C]-amorphigenin to 5 day old *A. fruticosa* seedlings, and re-isolation and degradation of the amorphigenin, have now shown that no movement of the label has occurred during these processes, confirming clearly that scrambling is a necessary part of the hydroxylation mechanism.

In the biosynthesis of amorphigenin, hydroxylation might occur earlier at the rot-2'-enonic acid stage and to assess this possibility [4'-^{3}H]-4'-hydroxyrot-2'-enonic acid (**4**) was administered in phosphate buffer (pH 8—9) and was satisfactorily (1.34%) incorporated into [8'-^{3}H]-amorphigenin (**5**): degradation[1] confirmed the labelled position as 8'. [4'-^{14}C]-5'-Hydroxyrot-2'-enonic acid (**6**) was then similarly administered (1.00% incorporation into amorphigenin) and by degradation 94% of the isotope was found to be at C-7' in the latter (**7**). Its geometrical isomer, [5'-^{14}C]-4'-hydroxyrot-2'-enonic acid (**8**), was also converted into amorphigenin (0.42%) and again, by degradation, the label was found to be 95% at C-7'. It follows that the cyclase enzyme is not showing (*Z*)/(*E*) specificity towards the substrate but is acting in a chemospecific manner towards an allylic methyl. Stereospecificity as regards the creation of the 5'-centre in (**2**) however is maintained. Both (**6**) and (**8**) (unlabelled) are in fact natural products, as was shown by the following experiment. [4'-^{14}C]-Rot-2'-enonic acid was administered to germinating *A. fruticosa* seedlings, and after 24 h worked up using 'cold' (**6**) and 'cold' (**8**) as carriers. After crystallisation to constant count a ^{14}C incorporation of 0.37% into the (*Z*)-isomer (**6**), and 0.46% into the (*E*)-(**8**), was found.

Further confirmation of the hydroxylation patterns was found by a competition experiment in which [4'-^{14}C]-(**3**) and [4'-^{3}H]-(**4**) (d.p.m. ratio 1 : 27) were administered in admixture to *A. fruticosa* with work-up after 48 h. The incorporation into amorphigenin was [^{14}C]: 1.07%, [^{3}H]: 0.5%,† and into

† An experiment in which [4'-^{14}C]-(**3**) and [4'-^{3}H]-(**4**) were administered in the d.p.m. ratio 1 : 5 gave incorporations into amorphigenin of [^{14}C]: 0.86% and [^{3}H]: 0.31%.

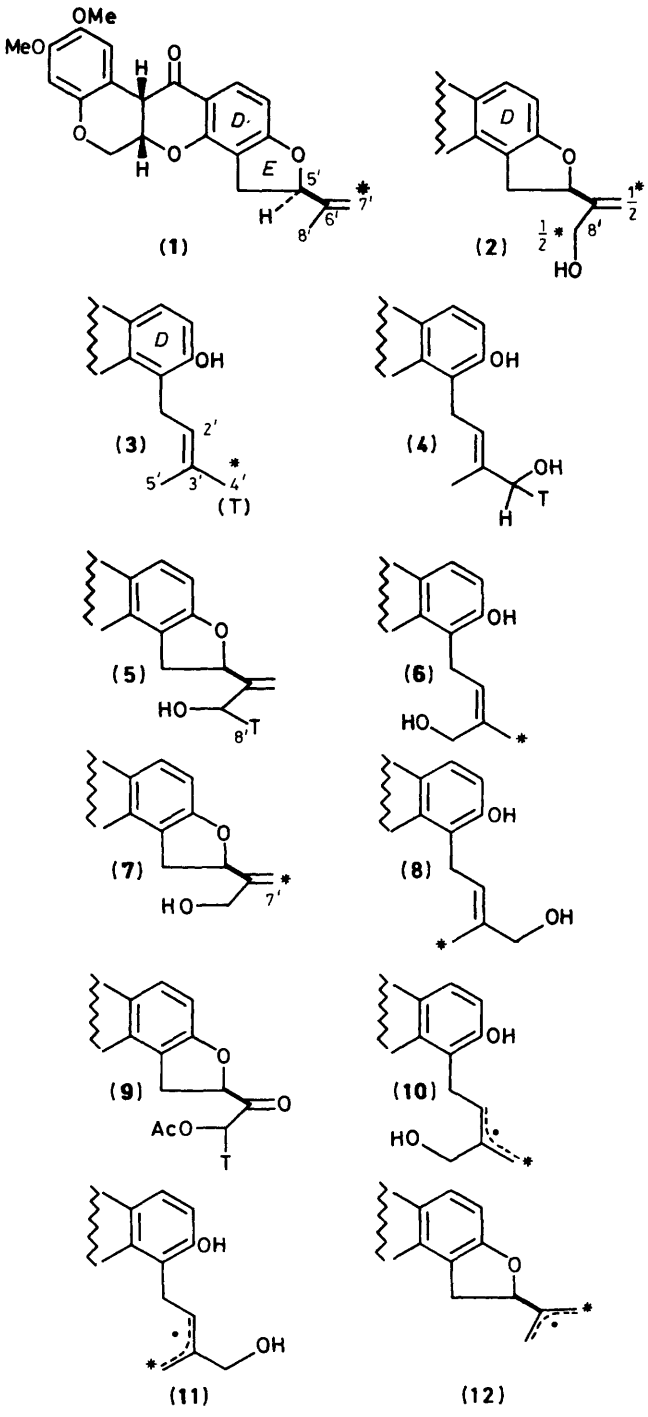


Scheme 1. Proposed grid for amorphigenin formation and labelling in *A. fruticosa*.

rotenone [^{14}C]: 0.84%, [^{3}H]: <0.002%. Degradation of the rotenone showed that all the [^{14}C]-label resided at C-7′ [double bond formation stereospecific, using the labelled (*E*)-methyl of rot-2′-enonic acid]. On the other hand <2% [^{3}H] was found at C-7′ in amorphigenin after acetylation at C-8′ and extraction of the 7′-methylene as formaldehyde: 98% of the [^{3}H] remained in the ketol acetate (**9**). The [^{14}C]-position in amorphigenin was very different, with 49% of the label recovered by degradation of the 7′-methylene to formaldehyde (dimedone) and 46% in the ketol-acetate (C-8′). Whereas [4′-^{3}H]-(**4**) has been converted into amorphigenin with specific label transfer to C-8′ of the latter, and not at all into rotenone, [4′-^{14}C]-(**3**) has been converted into rotenone with specific transfer to C-7′ of the latter and into amorphigenin with approximately even label scrambling between C-7′ and 8′.

Competitive administration of [7′-^{14}C]-rotenone (**1**) with [4′-^{3}H]-4′-hydroxyrot-2′-enonic acid (**4**) (d.p.m. ratio 1:6) to *A. fruticosa* with 48 h grow-on period resulted in amorphigenin carrying 1.26% of the original [^{14}C] and 0.21% of the original [^{3}H]. Though much smaller, the hydroxyrotenonic pathway is thus not negligible. An experiment in which [4′-^{14}C]-(**6**), and its tritiated geometrical isomer [4′-^{3}H]-(**4**), (d.p.m. ratio 1:5) were fed together to *A. fruticosa* gave amorphigenin carrying 0.22% of the original [^{14}C] and 0.22% of the original [^{3}H] indicating that (**4**) and (**6**) are equally acceptable precursors.

We conclude that whilst cyclisation of rot-2′-enonic acid (**3**) to give rotenone (**1**) in *A. fruticosa* is geometrically specific, with the (*E*)-methyl of the former becoming the 7′-methylene of the latter, the cyclisation of the geometrically isomeric hydroxyrotenonic acids (**6**) and (**8**) to amorphigenin is chemospecific for methyl groups of either geometry: radicals (**10**) and (**11**) for example might be involved. The hydroxylation processes in *A. fruticosa* are unspecific when operating on rotenone and on rot-2′-enonic acid. A radical mechanism may again be responsible *e.g.* (**12**) in the case of rotenone. Although the major route to amorphigenin is linear and *via* rotenone, the route by-passing rotenone is not negligible (~1/6th) and forms part of a grid process (Scheme 1) in 5 day old germinating seedlings: the relative importance of the two pathways may of course vary with age.



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References

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2 L. Crombie, *Nat. Prod. Rep.*, 1984, **1**, 3.