Biosynthesis of Rotenone and Amorphigenin. Study of the Origins of Isopropenyl-substituted Dihydrofuran E-Rings using Isotopically Labelled Late Precursors

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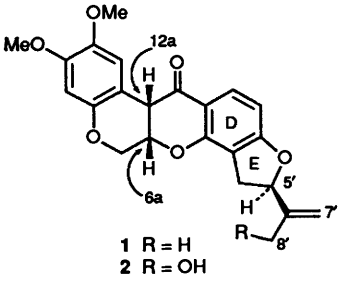
Whilst epoxidation of rot-2'-enonic acid is the most likely source of dalpanol in *Amorpha fruticosa* seedlings, administration of (5'*R*,6'S)-[7'-^{3}H]dalpanol shows that it is not an intermediate on the path to rotenone and amorphigenin. Labelled 4'-hydroxy- or 5'-hydroxy-rot-2'-enonic acid also do not qualify as intermediates in rotenone biosynthesis, but they are each converted into amorphigenin with chemospecific attack on the methyl group. By administration and re-isolation of [8'-^{14}C]amorphigenin from *A. fruticosa* seedlings, our earlier conclusion that hydroxylation of rotenone to form amorphigenin proceeds with even label scrambling between C-7' and C-8', probably *via* an allylic radical, is confirmed. Competitive double-labelling experiments are employed to support a scheme in which rotenone derives directly from rot-2'-enonic acid by an enzyme-induced radical-type reaction without the intervention of an hydroxylated intermediate, and the two labelled hydroxyrot-2'-enonic acids are similarly cyclised using their methyl groups. The incorporations into amorphigenin of labelled 4- and 5-hydroxyrot-2'-enonic acids, both of which are shown to occur naturally in *A. fruticosa*, are similar, but only about one sixth that of rotenone.

This, and our related biosynthetic work, rests on an extensive programme of isotopic labelling and reconstructive synthesis. Our earlier method for making [7'-^{14}C]-rotenone has been improved, and similar procedures adapted for [7'-^{13}C]- and [7'-^{14}C]-amorphigenin. 8'-Labelled rotenones are made by a positional interchange using addition of benzeneselenenyl chloride and elimination of the selenoxide, whilst [8'-^{14}C]amorphigenin is made *via* addition of phenylselenophthalimide. Unlabelled amorphigenin can be isotopically labelled by oxidation to the aldehyde and reduction using sodium borodeuteride or borotritide and a method additional to those we have described earlier is given for tritium labelling of rot-2'-enonic acid. [^{13}C]- and [^{14}C]-Labelling in the 5'-position of 4'- and 5'-hydroxyrot-2'-enonic acids can be attained through the catalytic hydrogenolysis of [7'-^{14}C]amorphigenin though special methods must be used to scrub the samples totally free from the latter. Methods based on the hydrolysis of labelled 4'-bromorot-2'-enonic acid are also described, and 4'-tritium-labelled 4'-hydroxyrot-2'-enonic acid is made from unlabelled material, or from rot-2'-enonic acid, by simple oxidation/reduction methods.

In the previous paper [1] we have described our results concerning the biosynthesis of the four-ring heterocyclic structures which constitute the rotenoid cores. We now turn to the prenylation processes which form the isopropenyl dihydrofuran E-ring of rotenone **1**, and of its 8'-hydroxy derivative amorphigenin **2**.[2] Owing to the very low incorporations of customary biosynthetic prenyl precursors, mevalonic acid, isopentenyl alcohol, dimethylallyl alcohol and 3-hydroxy-3-methylglutaric acid,[3] we have had to devise other ways of studying the formation of the E-ring structures of rotenone and amorphigenin in the plants *Derris elliptica* and *Amorpha fruticosa* where they are, respectively, the major rotenoids. These approaches have necessitated reconstructive synthesis of a number of chemospecifically and stereospecifically labelled test precursors mainly originating from rotenone and amorphigenin isotopically labelled on their 7'-methylene groups. The present paper is therefore divided into two sections. The first deals with the biosynthetic problem using labelled precursors, the second with the synthesis of these labelled compounds.

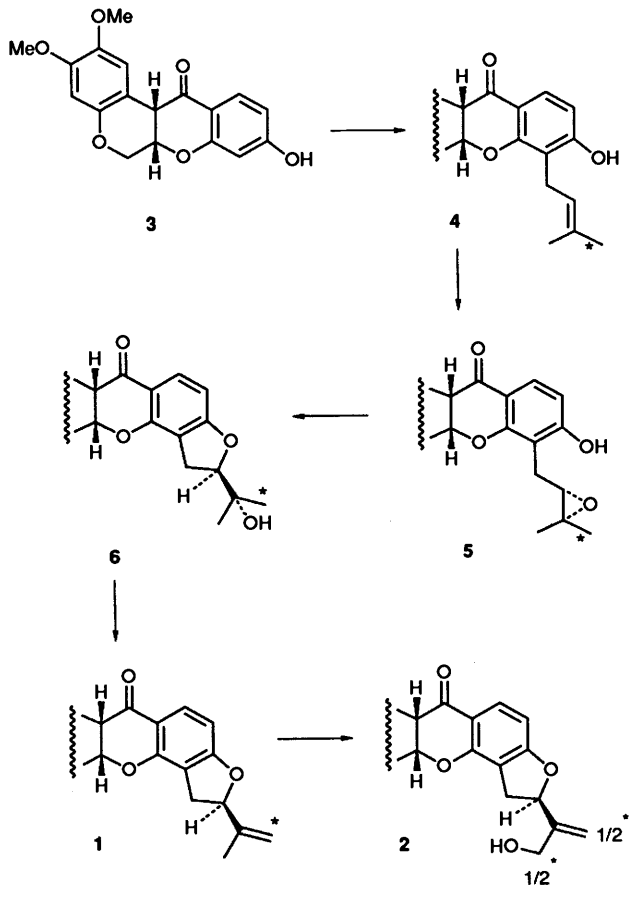


1 R = H
2 R = OH

Part 1. Biosynthesis of the Isopropenyldihydrofuran E-Rings of Rotenone and Amorphigenin

A plausible proposal for the biosynthesis of the ring-E of rotenone **1** and its 8'-hydroxy derivative amorphigenin **2** is shown in Scheme 1.[3] Starting from (6a*S*,12a*S*)-9-demethylmunduserone **3**,[4] rot-2'-enonic acid **4** is formed by prenylation and is then epoxidised to give the (2'*S*,3'*S*)-epoxide **5**. This configuration is required to generate the natural (5'*R*)-configuration in dalpanol **6**.[3] Dehydration would then have to occur by involving the *pro*-(*S*) methyl [derived from the (*E*)-methyl of rot-2'-enonic acid as shown by the stars] since it is known that the methylene of rotenone **1** is derived from the latter. Hydroxylation of rotenone at C-8' would then lead to amorphigenin which, according to our earlier experimental results, has an evenly scrambled label as between C-7' and C-8'.[3] The Scheme is plausible since the molecular structures **1**–**4** and **6** have been demonstrated to be present in *Amorpha fruticosa*, the germinating seed system we employ in our biosynthetic work on amorphigenin. It is also known that [4'-^{14}C]rot-2'-enonic acid is satisfactorily incorporated into

amorphigenin (0.77%) and [7′-^{14}C]rotenone is also well incorporated (0.57–1.89%).[3] It remained to show that dalpanol was a satisfactory intermediate, if the proposal were to be upheld.



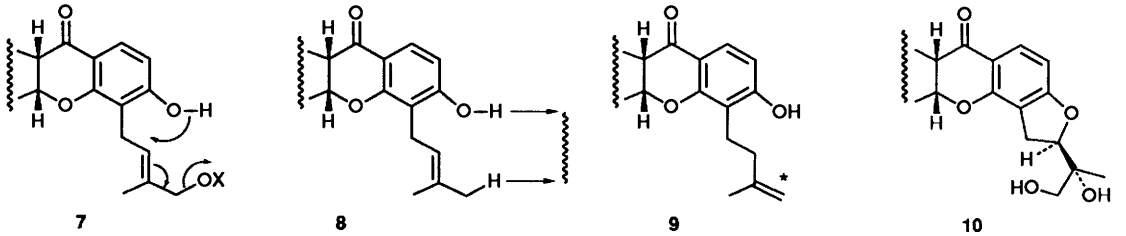
Scheme 1 Initial hypothesis for the formation of rotenone and amorphigenin *via* dalpanol

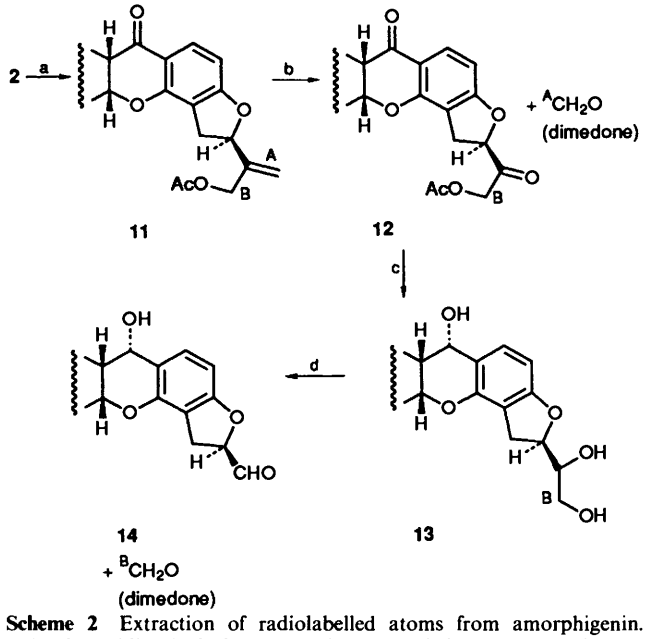
We have recently studied the labelling of the prochiral methyls of the hydroxyisopropyl group of dalpanol[5] and (6a*S*,12a*S*,5′*R*,6′*S*)[7′-^{3}H]dalpanol **6** was thus available for the incorporation experiments. The result showed clearly that dalpanol was not an intermediate for either rotenone or amorphigenin as in Scheme 1. Labelled dalpanol was not incorporated into either rotenone or amorphigenin when our standard *A. fruticosa* seedling was employed, nor when a crude enzyme system was applied. Wick feeding of *Derris elliptica* failed to produce incorporation into rotenone and so did administration to *Tephrosia vogellii* seedlings. Dalpanol is to be viewed as a terminus rather than an intermediate, though in our opinion it is probably formed *via* the epoxide as shown.

As a second hydroxylated candidate precursor, the labelled hydroxylated rot-2′-enonic acid (**7**; X = H) which might undergo cyclisation by an S_N2' elimination (perhaps with X = PP) was administered to *A. fruticosa* but again there was no incorporation into rotenone, though the position with regard to amorphigenin was different (see later). As in our related work on chromene formation[6,7] there is no evidence that hydroxylated intermediates are involved in these cyclisations and we favour a dehydrogenation pathway (structure **8**) from compound **4**. The oxidation is probably a radical process initiated by an enzyme similar to a P450 type, or deguelin cyclase, in which two hydrogen atoms are removed with attendant cyclisation. This mechanism is consistent with the fact that the isomer of compound **4**, [4′-^{14}C]rot-3′-enonic acid **9**, is not a precursor of amorphigenin, despite the fact that of all the small (C_5 or C_6) prenyl precursors tested by us earlier using the *A. fruticosa* system, [1-^{3}H]isopentenyl alcohol gave the best (though still poor) incorporation into amorphigenin.[3] In earlier work we have also shown that (6a*S*,12a*S*,5′*R*,6′*R*/*S*)-6′,7′-dihydroxy-[7′-^{14}C]rotenone (*cf.* structure **10**) is not a precursor for amorphigenin in *A. fruticosa*.[3]

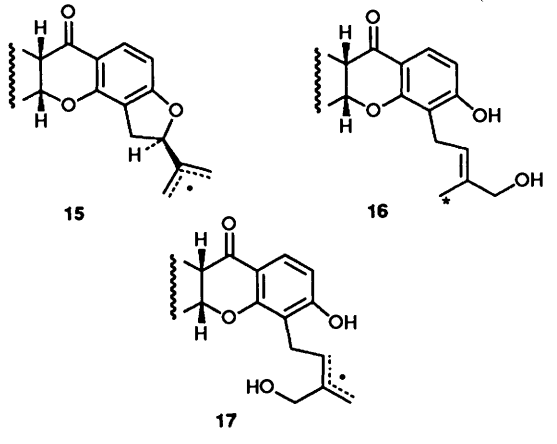
As mentioned above, both [4′-^{3}H]rot-2′-enonic acid and [7′-^{14}C]rotenone were satisfactorily incorporated into amorphigenin by *A. fruticosa* seedlings, but with approximately even scrambling of the ^{14}C label in the latter case between C-7′ and C-8′.[3] Owing to the possibility of inadvertently generating a symmetrical allyl species during work-up and degradation, three types of work-up were used but the conclusion was always that scrambling was part of the reaction mechanism. In order to put the matter beyond any doubt, however, we have designed an unequivocal experiment to settle the matter. [8′-^{14}C]Amorphigenin was synthesized and administered to 5-day-old *A. fruticosa* seedlings in the usual way. Amorphigenin was then re-isolated and degraded using our full standard degradation procedure (Scheme 2) to ascertain the position of the label. The ^{14}C-label remained almost entirely in the original 8′-position and showed no sign of scrambling, thus confirming our earlier conclusion that scrambling forms part of the actual hydroxylation mechanism. Probably an allylic radical (**15**) is involved.

It seemed possible that the origins of amorphigenin might be more complex than a straightforward hydroxylation of rotenone. Thus, whilst not leading to rotenone (above), hydroxylation at the rot-2′-enonic stage, followed by cyclisation, could provide an alternative route to amorphigenin. In order to assess this possibility, 4′-hydroxy[4′-^{3}H]rot-2′-enonic acid **18** was administered in phosphate buffer (pH 8–9) to *A. fruticosa* seedlings. A good incorporation of 1.34% into [8′-^{3}H]-amorphigenin **2** was attained, the position of the label in the latter being ascertained by degradation. The formaldehyde (isolated as the dimedone derivative) containing C-7′ was virtually inactive, all the radioactivity being confined to the keto acetate **12**. Recovery of C-8′ as formaldehyde (dimedone), however, accounted for only 71% of the original radioactivity but this is ascribed to tritium leakage through enolisation, a situation that does not occur with ^{14}C-labelling (see later). 5′-Hydroxy[4′-^{14}C]rot-2′-enonic acid **19** was then similarly administered, with 1.00% being incorporated into amorphigenin. By degradation 94% of the isotope was located in C-7′ of the latter which is therefore [7′-^{14}C]amorphigenin **2**. The geometric isomer of **19**, *i.e.* **16** was also administered in a parallel experiment to the *A. fruticosa* system. It was satisfactorily





Scheme 2 Extraction of radiolabelled atoms from amorphigenin. a, Ac_2O, pyridine; b, OsO_4, metaperiodate; c, $LiAlH_4$; d, metaperiodate.



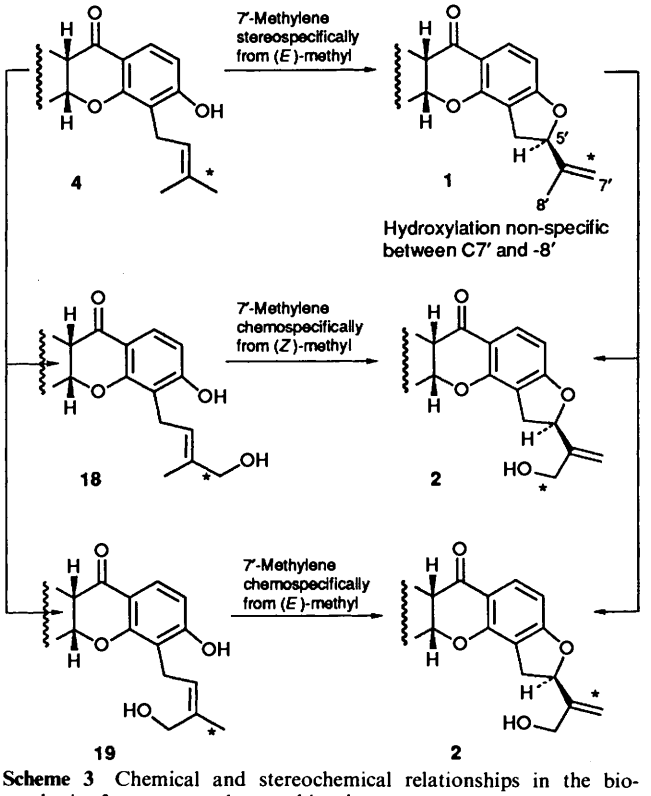
incorporated into amorphigenin (0.42%) and the product was again found to be [7′-^{14}C]amorphigenin by extraction and counting of C-7′ as formaldehyde dimedone. It follows that the cyclising enzyme involved is not showing a *Z*/*E* specificity towards its substrate, but is acting in a chemospecific manner towards the allylic methyl. Stereospecificity as regards the formation of the 5′-centre is, however, maintained.

The labelled samples administered, substrates **18** and **19**, were of reconstructed synthetic origin and might therefore have been treated by the enzyme as foreign substrates. In order to gain information as to the existence of compounds **18** and **19** (unlabelled) as natural products contained within the *Amorpha* system, [4-^{14}C]rot-2′-enonic acid was administered to *A. fruticosa* seedlings and after 20 h was worked up using 'cold' **18** and 'cold' **19** as carriers. After crystallisation to constant count, a ^{14}C-incorporation of 0.37% into the (*Z*)-hydroxy isomer **19** and 0.46% into the (*E*)-hydroxy isomer **18** was found. It follows that these two compounds are, in fact, natural products and may be presumed to be involved in the active metabolic system.

In order to obtain further confirmation of the above results, some competition experiments were initiated. [4′-^{14}C]Rot-2′-enonic acid **4** and 4′-hydroxy-[4′-^{3}H]rot-2′-enonic acid **18** were administered to the *Amorpha* system at a d.p.m. ratio of 1:27 with work-up after 48 h. The incorporation into amorphigenin was [^{14}C]: 1.07%; [^{3}H]: 0.54% and into rotenone [^{14}C]: 0.84%; [^{3}H]: <0.002%. Degradation of the labelled rotenone showed that all of the [^{14}C]-label resided at C-7′, confirming that double-bond formation was stereospecific, using the *E*-methyl of rot-2′-enonic acid and not the *Z*-methyl. On the other hand, <2% of the ^{3}H was found at C-7′ in amorphigenin after acetylation and extraction of the 7′-methylene as formaldehyde (dimedone). The remaining 98% of tritium was in the keto acetate. The [^{14}C]-position in amorphigenin was very different. In this case 49% of the total label was recovered by degradation of the 7′-methylene to formaldehyde and 46% remained in the keto acetate containing C-8′. Thus, whereas [4′-^{3}H]-**18** has been converted into amorphigenin with specific label transfer to C-8′ of the latter, no detectable transference into rotenone occurs. By contrast, [4′-^{14}C]-**4** is converted into rotenone with specific label transfer to C-7′ of the latter, and into amorphigenin with approximately even labelling scrambling between C-7′ and C-8′.

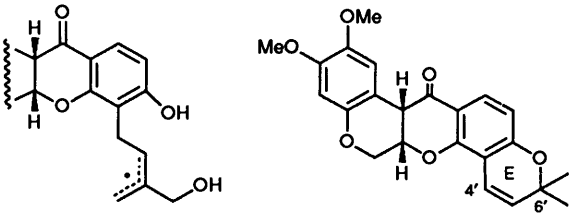
To gain some idea of the relative importance of the two pathways to amorphigenin in *A. fruticosa*, a competitive administration experiment between [7′-^{14}C]-rotenone **1** and 4′-hydroxy[4′-^{3}H]rot-2′-enonic acid **18** (d.p.m. ratio 1:6) was initiated. After a grow-on period of 48 h the amorphigenin isolated carried 1.26% of the original [^{14}C] and 0.21% of the original [^{3}H]. Whilst being less significant than the hydroxylation of rotenone, the hydroxyrot-2′-enonic acid pathway is by no means negligible. When 5′-hydroxy[4′-^{13}C]rot-2′-enonic acid **19** was administered to *A. fruticosa* along with 4′-hydroxy[4′-^{3}H]rot-2′-enonic acid (radioactivity ratio 1:5), an incorporation of 0.22% into amorphigenin was recorded for each, indicating that these two precursors are about equally acceptable substrates.

Scheme 3 summarises the results which have emerged from this investigation. The dehydrogenation and cyclisation of rot-2′-enonic acid is stereospecific with respect to the formation of the new chiral centre at C-5′, and the 4′(*E*)-methyl is used exclusively to form the 7′-methylene of rotenone. At least three pathways are available to form amorphigenin. In the first of these rotenone is hydroxylated at C-8′ to the extent of 50% with

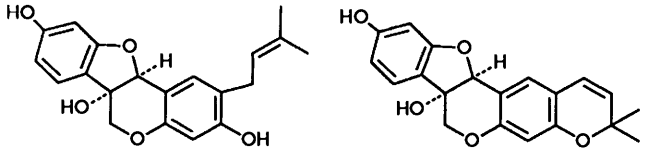


Scheme 3 Chemical and stereochemical relationships in the biosynthesis of rotenone and amorphigenin

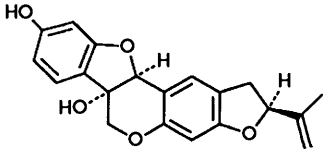
retention of the double-bond position at C-7′ together with its methylene label, and this process is accompanied by hydroxylation at labelled C-7′ with attendant migration of the double bond to C-8′, the process occurring to give 50% labelling at each centre. It therefore implicates a symmetrical intermediate in the hydroxylation, probably an allylic radical. The other two processes involve hydroxylation at the rot-2′-enonic acid stage. 4′(*E*)-Labelled 4′-hydroxyrot-2′-enonic acid cyclises with dehydrogenation into the unlabelled 5′(*Z*)-methyl. On the other hand 4′(*E*)-labelled 5′-hydroxyrot-2′-enonic acid also cyclises with dehydrogenation but into the now labelled 4′(*E*)-methyl, showing that the cyclisation is chemospecific for the methyl group. Both cyclisations are stereospecific with respect to the formation of the 5′(*R*) centre of amorphigenin. It is not known whether in Nature compounds **18** and **19** are formed in equal amounts but it seems reasonable that cyclisation of the hydroxyrot-2′-enonic acids should involve delocalised allylic radicals **17** and **20**, formation of 5-membered rings being preferred to that of 7-membered ones.



20 **21**



22 **23**



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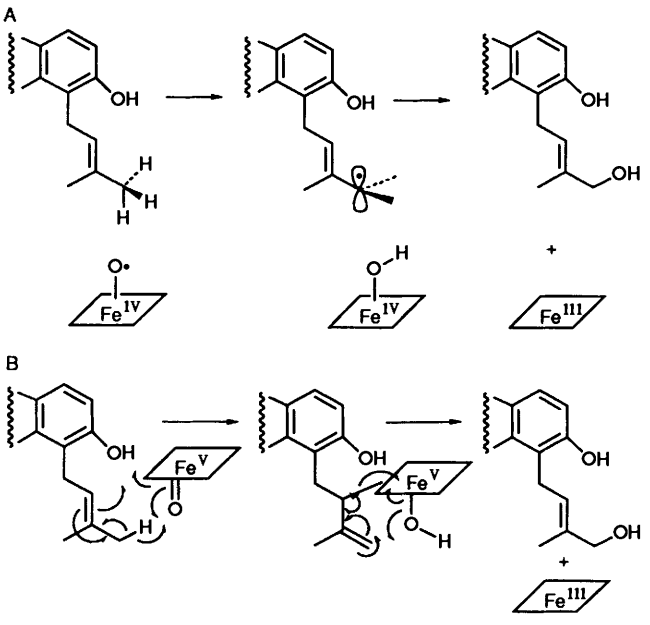
Whilst a soluble iron-containing enzyme which converts rot-2′-enonic acid into the chromene deguelin **21** in *Tephrosia vogellii* has been discovered and isolated in our laboratory,[8,9] the dehydrogenating and cyclising enzyme(s) which forms rotenone/amorphigenin has not been isolated from *A. fruticosa*, though it would appear to be of a similar type. However, Welle and Grisebach[10] have reported that a microsome preparation from elicitor-challenged soya bean cell suspension culture contained an enzyme(s) which could cyclise the prenylated pterocarpan **22** to glyceollin II **23** and III **24**, the reaction to form the latter being similar in type to the conversion of rot-2′-enonic acid into rotenone. The enzyme(s) was characterised as a P450 monooxygenase and there was no evidence of oxygenated intermediates. Being an enzyme of the endoplasmic reticulum it differs from our soluble *T. vogellii* enzyme[9] and was unable to bring about cyclisation of rot-2′-enonic acid to form rotenone or deguelin.

The enzyme responsible for the allylic hydroxylation of rotenone and rot-2′-enonic acid is likely to be a cytochrome P450 monooxygenase and two possible mechanisms have been suggested (Scheme 4).[11] One (A) involves insertion of the oxygen atom into the C–H bond of the C-7 methyl *via* a radical of indeterminate lifetime. The other (B) suggests a regiospecific two-step mechanism involving an ene reaction followed by a [2,3]-sigmatropic rearrangement. Our results, particularly those concerning the non-specific hydroxylation of rotenone, are clearly more consistent with mechanism (A).

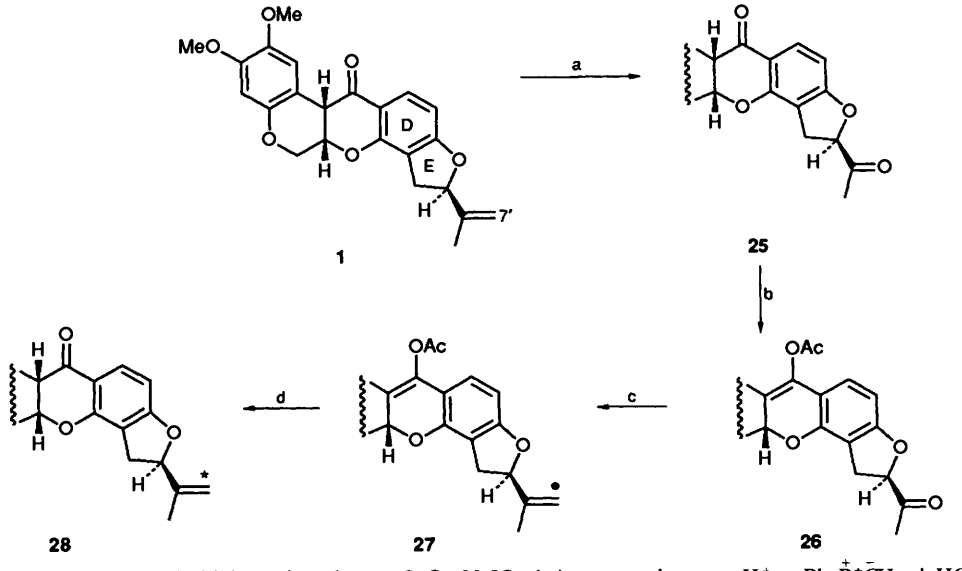


Scheme 4 Proposals for enzyme allylic hydroxylation

Part 2. Reconstructive Synthesis of Late Precursors for Study of Rotenoid Biosynthesis

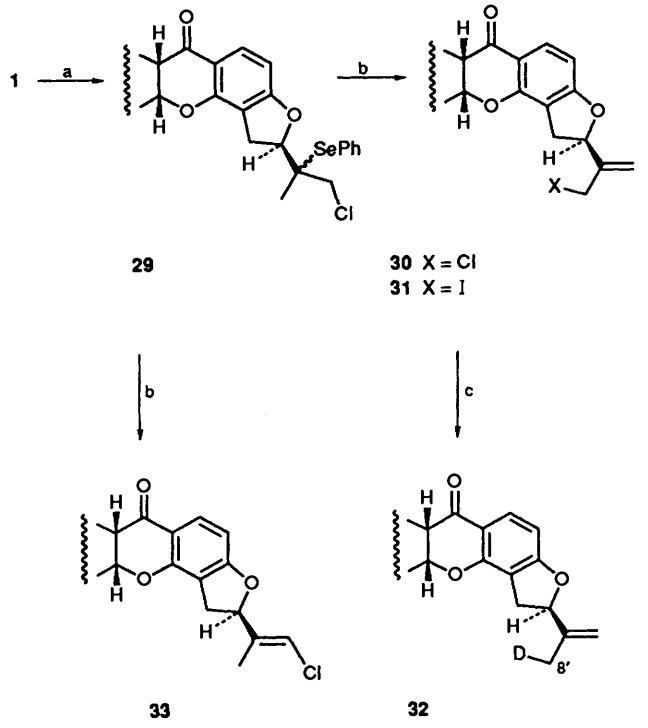
Rotenone carrying an isotopic label in the 7′-methylene is a key starting material in many of the chemical and stereochemical transformations required and this is considered first.

(6aS,12aS,5′R)-[7′-^{14}C]*Rotenone* **28**.—Our earlier method[12] for making the latter employed trimethylsiloxy blocking of the base-sensitive and enolisable 12a-position in rotenone, thus preventing the easily induced β-elimination which racemises C-6a. This was followed by excision of the 7′-methylene, replacement by a 7′-labelled (^{14}C, ^{13}C or ^{3}H) methylene, and finally deblocking at C-12a. We have now improved and simplified the procedure by using enol acetate blocking as indicated in Scheme 5. The diketone **25** is readily available by standard osmium tetraoxide/periodate cleavage.[13] Treatment of the diketone with isopropenyl acetate and sulfuric acid, a process which does not racemise C-6a,[14] gave the crystalline enol acetate **26** without attack on the E-ring appendage. Wittig coupling of the latter using 'cold' methylenetriphenylphosphorane in tetrahydrofuran (THF) gave a low yield of product (~20%) which was considerably improved by using 2 mol equiv. of ylide (~55%), though this would be wasteful of labelled material. Examination of a 'cold' reaction run for a short time (15 min) and quenched with D_2O gave recovered acetate **26** heavily deuteriated on the C-7′ methyl (NMR and mass spectral evidence), though there was no evidence of epimerisation at C-5′. Adjustment of reaction conditions (small volume of THF, rapid syringe addition of the ketone, shortened reaction time) allowed methylenation without epimerisation at C-5′ to give isopropenyl compound **27** in ~45% yield. Mild acid hydrolysis regenerated (6a*S*,12a*S*,5′*R*)-rotenone. In this way [7′-^{14}C]rotenone **28** (Scheme 5) was made in an overall chemical yield of 30%, and a radiochemical yield of 33% based on [^{14}C]methyltriphenylphosphonium iodide. Chemical degradation of the [^{14}C]rotenone to formaldehyde (dimedone) and rotenone 6′-norketone showed the labelling to be entirely confined to the methylene group.



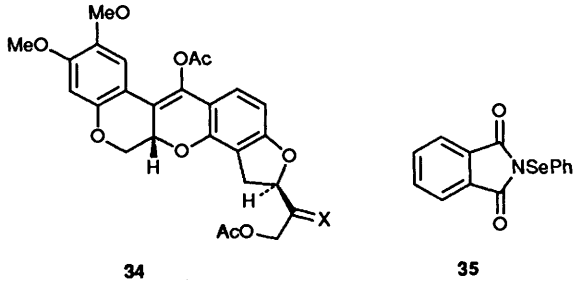
Scheme 5 Labelling of rotenone at C-7′ with isotopic carbon. a, OsO_4, $NaIO_4$; b, isopropenyl acetate, H^+; c, $Ph_3\overset{+}{P}{}^*\overset{-}{C}H_2$; d, HCl, MeOH.

(6aS,12aS,5′R)-[8′-^{2}H]*Rotenone* **32**.—Natural rotenone was treated with benzeneselenenyl chloride[15] in THF to give the addition product **29**. The pair of distereoisomers formed displayed ^{1}H NMR signals at δ 1.1 and 1.25, indicating anti-Markownikov addition *via* kinetically controlled halide attack on the bridged ion.[16] This product was converted into the selenoxide, which underwent ready elimination to give a mixture of the allylic chloride **30** and the vinyl chloride **33** (3:1). These could be separated chromatographically (HPLC) or by crystallisation from ethyl acetate or chloroform–methanol. Conversion of the allylic chloride into the iodide **31** by Finkelstein's method, followed by treatment with sodium cyanoborodeuteride in hexamethylphosphoric triamide (HMPA) for 2 h at 65 °C, inserted a deuterium atom at C-7′ to give the title product **32** without affecting either the C-12 carbonyl or causing any racemisation of the C6a–C12a bridge (Scheme 6). The deuterium NMR spectrum showed the expected broad triplet at δ 1.79. By starting from 7′-labelled rotenone, the label can also be translated to C-8′.



Scheme 6 Labelling of rotenone at C-8′ with deuterium. a, PhSeCl; b, H_2O_2; c, sodium cyanoborodeuteride.

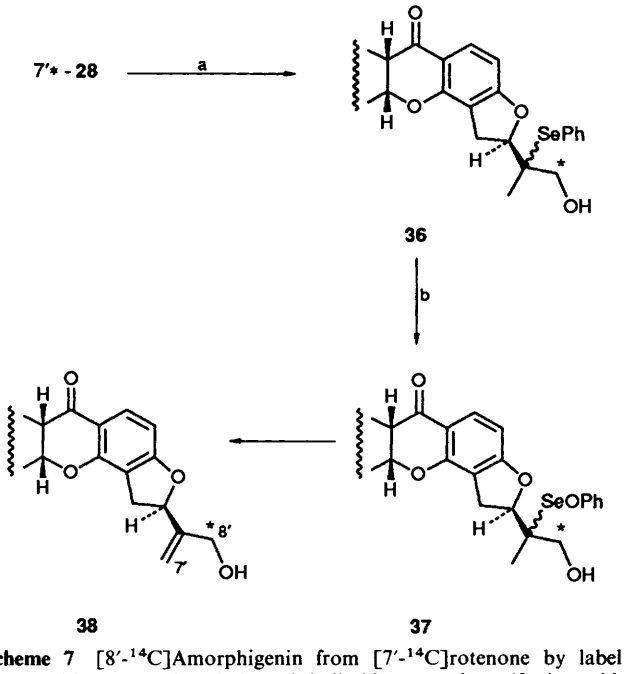
(6aS,12aS,5′R)-[7′-^{13}C]- *and* -[7′-^{14}C]-*Amorphigenin* **2**.—Amorphigenin was labelled by a procedure similar to that used for rotenone, the Wittig reaction being carried out on the bisacetyl derivative **34** (X = O), both acetates being finally removed by acid hydrolysis to give 7′-labelled amorphigenin **2**. Both [^{13}C]- and [^{14}C]-labelled methylenetriphenylphosphoranes were used. Examination of the [^{13}C]-product by ^{13}C NMR spectroscopy showed that the ^{13}C-label was entirely located at C-7′.



(6aS,12aS,5′R)-[8′-^{14}C]*Amorphigenin* **38**.—This was achieved by a process of label transposition. [7′-^{14}C]Rotenone **28** was treated for 65 h with *N*-phenylselenophthalimide **35**,[17] an efficient phenylseleno carrier, in dichloromethane containing a catalytic amount of camphorsulfonic acid and 2–3 mol equiv. of water to form the β-hydroxy selenide **36**. The reaction probably proceeds *via* a bridged ion. Treatment with hydrogen peroxide then gave the selenoxide **37**, which eliminated readily to give the desired [8′-^{14}C]amorphigenin. Initially overall yields of amorphigenin were poor though examination of the reaction by NMR spectroscopy after 65 h showed that the β-hydroxy selenide **37** was formed in >90% yield. Although oxidative elimination of β-hydroxy selenides gives secondary and primary alcohols in good yields, the usual method gives poor results for primary alcohols and the addition of alumina has been suggested.[18] A recommended procedure using *tert*-butyl hydroperoxide[18] was not effective in our case but 30% H_2O_2–THF–Al_2O_3 at 35 °C gave a good yield of amorphigenin. Unfortunately the basic conditions racemised the B/C ring junction. Considerable experimentation was involved to find suitable

conditions which employ pyridine (20 mm^3 mg^{-1} of rotenone) over a period of 2 h (see Experimental section). This gave unracemised amorphigenin in reproducible yields of 36%. Chemical degradation showed that >91% of the label was recovered from C-8′ extracted as formaldehyde dimedone but 7.5% was also found in the formaldehyde derivative from C-7′, indicating that the labelling is not completely specific for C-8′. A small correction factor is required for its use in certain experiments.

In a further attempt to improve the reaction the enol acetate of rotenone (compound **27**) was used in place of rotenone. The required β-hydroxy selenide was formed in ~80% yield. Oxidation with the H_2O_2–THF–Al_2O_3 reagent at 55 °C gave amorphigenin enol acetate in 50% yield and mild acid hydrolysis returned the 6a*S*,12a*S* configuration, giving amorphigenin **38** in 67% yield, 34% overall (Scheme 7). Since rotenone is available labelled at C-7′ with ^{13}C, ^{2}H or ^{3}H the methods are applicable to other isotopic situations.



Scheme 7 [8′-^{14}C]Amorphigenin from [7′-^{14}C]rotenone by label transposition. a, Phenylselenophthalimide, camphorsulfonic acid, water; b, H_2O_2.

We have shown in earlier work that amorphigenin can be oxidised to the 8′-aldehyde by manganese dioxide.[19] Reduction of the aldehyde therefore gives a potential method for the introduction of [8′-^{2}H]- or [8′-^{3}H]-labelling. However, experiments with sodium borohydride showed that besides the 8′-aldehyde the 12-carbonyl was also partly reduced, giving a low (24%) yield of amorphigenin. The situation was not improved when sodium triacetoxyborohydride or sodium trimethoxyborohydride was used in the reduction. Since natural amorphigenin is a more scarce material than rotenone, the method above was preferred for labelling purposes.

(6aS,12aR)-(E)-[4′-^{14}C]-, -[4′-^{13}C]-, -[4′-^{3}H]- *and* -[4′-^{2}H]-*Rot-2′-enonic Acid* **40**.—The first two of these were made by treatment of the appropriately labelled specimens of rotenone with boron tribromide (1 mol equiv.) to give the (*E*)-bromide **39** as described earlier.[12] Reduction with sodium cyanoborohydride in HMPA causes reduction of the halogen without attack on the carbonyl group, giving the rot-2′-enonic acid **40** labelled at C-4′.[12] When the boron tribromide reaction was carried out with 'cold' rotenone to give 'cold' 4-bromorotenonic acid and then reduction effected with sodium cyanoborodeuteride or cyanoborotritide (*E*)-[4′-^{2}H]- and -[4′-^{3}H]-rot-2′-enonic acids **41** and **42** were also made.

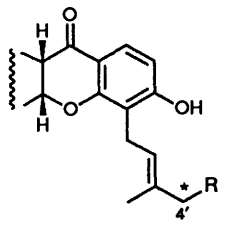
In our earlier work we also made (*E*)-[4′-^{13}C]- and -[4′-^{14}C]-rot-2′-enonic acids by heterogeneous catalytic (Pd/C) hydrogenolysis in pyridine.[12,20] Some rot-3′-enonic acid is also formed and must be separated, but the main disadvantage is that the hydrogenolysis is not entirely stereospecific, the (*E*)-[^{13}C]- or (*E*)-[^{14}C]-product containing 10–20% of (*Z*)-material.[12] Although suitable for experimental use, greater inaccuracies are involved unless each batch is carefully analysed and corrections made for the (*Z*)-impurity.

(6aS,12aS)-(Z)-5′-*Hydroxy*-[4′-^{13}C]- *and* -[4′-^{14}C]-*rot-2′-enonic Acid* **19** *and* (6aS,12aS)-(E)-4′-*Hydroxy*-[5′-^{13}C]- *and* -[5′-^{14}C]-*rot-2′-enonic Acid* **16**.—An examination of the catalytic hydrogenolysis of unlabelled amorphigenin showed that there were differences relative to the rotenone case. The products after the addition of 1 mol of hydrogen were compounds **43**, **16** and **19** in the approximate proportions 2:1:1, thus showing different behaviour relative to the rotenone case: addition of further hydrogen saturated the double bond. The ~1:1 ratio of the latter two compounds, which were particularly required to test as biosynthetic intermediates, was consistent in subsequent unlabelled experiments, the substantial selectivity found in the case of rotenone not being present. Separation of the product mixture could be effected readily by chromatography and an experiment using [7′-^{13}C]amorphigenin gave the labelling pattern shown on structures **43**, **16** and **19**. The experiment was then carried out using [7′-^{14}C]amorphigenin to give the [^{14}C]-samples used in our biosynthetic work. It seems possible that the difference found between the hydrogenolyses of rotenone and amorphigenin lies in the hydrogen-bonded structure **44** which influences the formation of more (*E*)-labelled material.

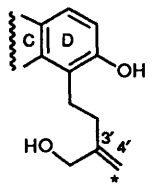
Our experiments on the preparation of 4′-tritium-labelled rot-2′-enonic acid by tritiolysis of rotenone have shown that the use of palladium catalyst and tritium leads to extensive, unwanted exchange of hydrogens in the rotenoid core, particularly the 6a,12a-bridge, and the method is unsuitable.

Other ways of making carbon-labelled 4′-hydroxyrot-2′-enonic acid **18** were also devised. Solvolysis of the allylic bromide **39** in acetone–water at 60 °C gave compound **18** in 56% yield, though in the presence of sodium hydrogen carbonate participation of the 9-hydroxy groups occurs giving rotenone as a mixture of epimers at C-5′. Silver oxide in acetone at 20 °C provided alternative conditions (65% yield). As a check on possible racemisation at C-6a, -12a, the latter sample was converted into the tetrakismethyl ether and had exactly the same rotation as an authentic specimen.[12] When the bromide was converted into the alcohol by using silver acetate in acetic acid, followed by gentle hydrolysis with potassium carbonate in methanol–water; however, extensive racemisation was shown by conversion into the tetrakismethyl ether. Though wasteful on label, racemised, or partially racemised, material would be adequate for some biological experiments.

Whilst the NMR spectra for the 4′- and the 5′-hydroxyrot-2′-enonic acids are very similar, there is an interesting feature that deserves comment. In the proton spectrum of 4′-hydroxyrot-2′-enonic acid the signals for the two protons at C-4′ (δ 3.70) are partly obscured by one of the methoxy peaks (δ 3.69). However, evidence from the tritium NMR spectrum of 4′-hydroxy-[4′-^{3}H]rot-2′-enonic acid (see later) shows the resonances for these two diastereotopic (remote chirality at C-6a, -12a) protons to be very close to each other. In the case of 5′-hydroxyrot-2′-enonic acid, the two 5′-protons show a clear AB pattern (*J* 11.8 Hz) and are shifted downfield relative to the 4′-case by ~0.52 ppm. A possible explanation is restricted rotation due to hydrogen bonding placing the two protons in



39 R = Br
40 R = H (≡4)
41 R = D
42 R = T



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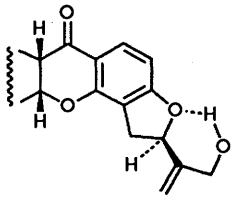

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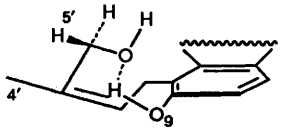

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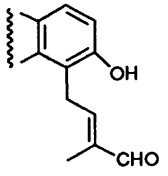

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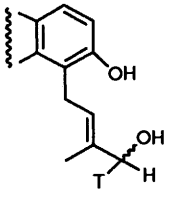

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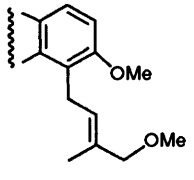

48

markedly distinct environments. Thus the 5′-hydroxy compound (but not the 4′-hydroxy one) can adopt a conformation in which there is hydrogen bonding between the 4′-hydroxy and the phenolic hydroxy groups, *e.g.* structure **45**, in which the phenolic hydroxy group is represented as the donor. There is also IR evidence of hydrogen-bonding differences. The 4′-hydroxy compound has a sharp band at 3530 cm^{-1} characteristic of an unbonded OH, whilst the 5′-hydroxy compound shows a relatively sharp intense band at 3430 cm^{-1} (coincident with a second, broader band) indicating hydrogen bonding.

The samples used in the biosynthetic work are derived from radioactive amorphigenin and deductions are drawn from counting amorphigenin after passage through the plant. It follows that the radioactive derivatives used in the administration must be scrupulously free from contaminating radioactive amorphigenin. Radioactive samples are too small for repeated recrystallisation and so the following purging technique was adopted. Each hydroxy compound was mixed with excess (about 20×) of unlabelled amorphigenin and the hydroxy compound and the amorphigenin were separated by preparative TLC (PLC) [silica; two developments with chloroform–propan-2-ol (20:1 v/v)]. The amorphigenin was then counted and the procedure was repeated. Not until the amorphigenin count fell to a negligible value (usually about four treatments) was the hydroxy compound accepted for a feeding experiment.

(6aS,12aS)-(E)-4′-*Hydroxy*-[4′-^{3}H]*rot*-2′-*enonic acid* **18**.—The allylic alcohol (**18**, unlabelled) was oxidised cleanly by pyridinium dichromate (PDC) in dimethylformamide (DMF) to give the aldehyde **46** (65%) (δ_H 9.31): oxidation with MnO_2 was also satisfactory. The same aldehyde could also be obtained (68%) by stereoselective oxidation of rot-2′-enonic acid by selenium dioxide.[21,22] Reduction with sodium borohydride (1 mol equiv.) in diglyme selectively reduced the aldehyde preferentially at the ketone group, in this case giving alcoholic compound **47** (unlabelled) (≡**18**, unlabelled) in 78% yield. A similar experiment was carried out with sodium borotritide. ^{3}H NMR spectroscopy of the product **47** showed two clean signals, at δ 3.70 and 3.72, arising from the (*R*) and (*S*) tritons at C-4′ which are in a diastereotopic situation because of the chirality at C-6a, -12a.

By treatment of 4′-hydroxy-[4′-^{3}H]rot-2′-enonic acid with triphenyl phosphite methiodide and sodium cyanoborohydride in HMPA, reduction of the hydroxy group is achieved,[23] giving a further source of [4′-^{3}H]rot-2′-enonic acid, additional to our other methods.

Experimental

Details of the seedling system, isotopic tracer administration, and work-up of *Amorpha fruticosa* are given elsewhere.[1,3] ^{14}C and ^{3}H Radioactivity was measured by using an Intertechnique SL 3000 liquid scintillation counter. All samples were counted in duplicate in Nuclear Enterprises NE250 fluid (10 cm^3) in plastic vials. HPLC was carried out using a Waters system, mainly with RAD PAK (10 cm × 8 mm) columns and UV and RI detection. NMR spectra were recorded using Bruker AM400, WM250 and WP80SY spectrometers, usually for solutions in $CDCl_3$. For ^{1}H and ^{13}C spectra a deuterium lock was employed. For ^{2}H spectra a fluorine lock or an unlocked field was used as appropriate and spectra are usually for solutions in $CHCl_3$. *J*-Values are given in Hz. IR data are for KBr discs. Units for specific optical rotations $[\alpha]_D$ are now given in units of 10^{-1} deg cm^2 g^{-1}. Light petroleum refers to the fraction boiling in the range 60–80 °C. UV data refer to ethanol solutions.

Biosynthetic Experiments

General Procedure for the Isotopic Label Extraction from Amorphigenin Derived from Administration Experiments to Amorpha fruticosa.—The following is a typical example.

Acetylation of the extracted amorphigenin. A solution of amorphigenin (99.3 mg, 0.242 mmol) in acetic anhydride (18 cm^3) and dry pyridine (4 drops) was stirred at room temperature (16 h) and poured into ice–water. The product was thoroughly extracted with chloroform and the extract was washed successively with aq. sodium hydrogen carbonate and water, then dried (sodium sulfate), and evaporated. The amorphigenin acetate **11** was further purified by PLC on silica with diethyl ether–ethyl acetate–light petroleum (5:2:2 v/v). The product (92 mg, 84%), m.p. 163–165 °C (lit.,[19] 166–167 °C), was crystallised to constant radioactive count from chloroform–methanol.

Osmium tetraoxide/sodium metaperiodate cleavage of amorphigenin acetate. A solution of amorphigenin acetate **11** (68.2 mg, 0.151 mmol) in a mixture of THF (3.5 cm^3) and water (1.5 cm^3) was treated with a mixture of osmium tetraoxide (5 mg) in *tert*-butyl alcohol (0.5 cm^3), and after the mixture had been stirred for 10 min sodium metaperiodate (140 mg, 0.66 mmol) was added, and the mixture was stirred for 3.5 h at 20 °C in a stoppered flask. The mixture was then diluted with water (20 cm^3) and carefully distilled into an aq. solution of dimedone reagent (60 cm^3; 3 g dm^{-3}). The dimedone solution was kept at 20 °C for 1 h and then overnight at 0 °C. The black mixture was filtered, the residue was dried (20 °C) and then taken up into chloroform, and the mixture was filtered through Kieselguhr. The colourless filtrate was evaporated to yield methylene bisdimedone (33 mg, 75%). The sample was crystallised to constant radioactivity from ethanol, m.p. 190 °C (lit.,[3] 190 °C). All water used was triply distilled, and this applies to similar work below.

The distillation residue was extracted with chloroform and the extracts were washed with aq. sodium hydrogen carbonate, dried (sodium sulfate), and evaporated. Purification by PLC on silica with light petroleum–chloroform–propan-2-ol (5:9:1) gave the keto acetate **12** (58 mg, 85%), which was crystallised to constant radioactivity from chloroform–diethyl ether, m.p. 195–197 °C (lit.,[3] 195–197 °C).

Reduction of the keto acetate **12** *with lithium aluminium hydride.* A solution of the keto acetate **12** (50 mg, 0.11 mmol) in anhydrous THF (5 cm^3) was added under nitrogen to a slurry of lithium aluminium hydride (140 mg) in dry THF (2 cm^3). After being stirred (3 h at 20 °C) the reaction mixture was quenched with saturated aq. ammonium chloride and extracted with chloroform. Work-up in the usual way gave the unstable triol **13** as a foam (47.3 mg). It was normally used immediately in the next stage though it could be purified by PLC on silica with chloroform–propan-2-ol (4:1).

Oxidation of the triol **13** *with sodium metaperiodate.* The triol **13** (47.3 mg), THF (3.0 cm^3), and water (1 cm^3) was stirred with sodium metaperiodate (94 mg) at room temperature in a stoppered flask for 3 h. Water (20 cm^3) was added and the formaldehyde solution was distilled into dimedone reagent (60 cm^3; 3 g dm^{-3}) leaving the hydroxy aldehyde **14**. After storage for 1 h at 20 °C and then overnight at 0 °C, the methylene bisdimedone was filtered off, dried (27.8 mg), and crystallised from ethanol to constant radioactive count, m.p. 189–190 °C.

Administration of [7′-^{3}H]*Dalpanol to* A. fruticosa, Derris elliptica *and* Tephrosia vogellii.—The dalpanol **6** carrying 2.26×10^8 dpm (specific activity 37.5 mCi $mmol^{-1}$) was administered to 300 *A. fruticosa* seedlings, which were then grown on for 48 h. An uptake of 54.0% was measured. The dpm of the amorphigenin recovered from the experiment, initially 9.25×10^4, sank to 418 after 6 crystallisations whilst the rotenone, initially 2.23×10^2, sank to 88 dpm after 4 crystallisations. In a second experiment [7′-^{3}H]dalpanol carrying 2.54×10^7 dpm was administered to 300 *A. fruticosa* seedlings. Uptake was 80.5%. The amorphigenin produced counted at 138 dpm, the rotenone at 33, both after 3 crystallisations. The experiments were repeated several more times, and also administered to *T. vogellii* seedlings which produce rotenone, but all experiments showed negligible incorporations.

Wick feeding of [7′-^{3}H]dalpanol (2.13 mg, 0.32 mCi mg^{-1}) to a 9 month old cutting of *D. elliptica*, growing on for 10 days, gave rotenone with a maximal incorporation of 0.001% after 2 crystallisations.

Administration of [8′-^{14}C]*Amorphigenin to* A. fruticosa.—The radioactive amorphigenin carrying 2.28×10^6 dpm was fed to 300 *A. fruticosa* seedlings and after a grow-on period of 48 h the amorphigenin was recovered from the plant (11%). It was acetylated and had a specific activity of 6.44×10^5 dpm $mmol^{-1}$. Degradation as above gave the 7′-methylene as formaldehyde dimedone (specific activity 2.21×10^3 dpm $mmol^{-1}$; 3.3% of the label) and the keto acetate (specific activity 6.45×10^5 dpm $mmol^{-1}$; 96.7% of label).

Formation of Amorphigenin by Administration of 4′-*Hydroxy*-[4′-^{3}H]*rot-2′-enonic Acid* **18** *to* Amorpha fruticosa *Seedlings.*—The radioactive precursor, sufficient to provide 4.08×10^8 dpm, was administered to 300 seedlings at a specific activity of 1.97×10^{11} dpm $mmol^{-1}$. Uptake into the seedlings was 62% of that administered. Amorphigenin (27.2 mg) was isolated having a total activity of 3.40×10^6 dpm, giving an incorporation of 1.34% at a specific activity of 5.13×10^7 dpm $mmol^{-1}$, dilution 3840.

Degradation of the amorphigenin at a specific activity of 1.93×10^6 dpm $mmol^{-1}$ (activity taken as unity) gave amorphigenin acetate [relative activity (RA) 1.03], 7′-formaldehyde dimedone (RA 0.002), keto acetate (RA 1.01) and 8′-formaldehyde dimedone (RA 0.71).

Formation of Amorphigenin by Administration of 4′-*Hydroxy*-[5′-^{14}C]*rot-2′-enonic Acid* **16** *to* A. fruticosa *Seedlings.*—The radioactive precursor, sufficient to provide 6.96×10^7 dpm, was administered to 300 seedlings at a specific activity of 1.05×10^{10} dpm $mmol^{-1}$. Uptake was 64%. Amorphigenin (52.3 mg) was isolated having a total activity of 1.86×10^5 dpm, giving an incorporation of 0.42% at a specific activity of 1.46×10^6 dpm $mmol^{-1}$, dilution 7192.

Degradation of the amorphigenin at a specific activity of 8.32×10^5 dpm $mmol^{-1}$ (activity taken as 1.00) gave amorphigenin acetate (RA 1.02), 7′-formaldehyde dimedone (RA 0.95) and the keto acetate (RA 0.009).

Formation of Amorphigenin by Administration of 5′-*Hydroxy*-[4′-^{14}C]*rot-2′-enonic Acid* **19** *to* A. fruticosa *Seedlings.*—The radioactive precursor sufficient to provide 8.35×10^7 dpm was administered to 300 seedlings at a specific activity of 1.05×10^{10} dpm $mmol^{-1}$. The uptake was 74%. Amorphigenin (50.4 mg) was isolated having a total activity of 6.16×10^5 dpm, giving an incorporation of 1.00% at a specific activity of 5.02×10^6 dpm $mmol^{-1}$, dilution 2092.

Degradation of the amorphigenin at a specific activity of 2.92×10^6 dpm $mmol^{-1}$ (activity taken as 1.00) gave amorphigenin acetate (RA 1.05), 7′-formaldehyde dimedone (RA 0.94) and the keto acetate (RA 0.002).

Formation of Amorphigenin and Rotenone by Competitive Administration of [4′-^{14}C]*Rot-2′-enonic Acid* **4** *and* 4′-*Hydroxy*-[4′-^{3}H]*rot-2′-enonic Acid* **18** *to* A. fruticosa *Seedlings.*—The [^{14}C]rotenonic acid **4**, sufficient to provide 3.23×10^6 dpm at a specific activity of 1.06×10^9 dpm $mmol^{-1}$, along with the hydroxy[^{3}H]rotenonic acid **18**, sufficient to provide 8.86×10^7 dpm (specific activity 9.8×10^{10} dpm), were administered together to 575 *A. fruticosa* seedlings, allowing for 48 h growth. Uptake of substrate **4** was 69.1% and that of substrate **18** 49.5%. Amorphigenin (42 mg) was isolated having a ^{14}C-activity of 2.43×10^5 dpm $mmol^{-1}$, giving an incorporation of 1.07%, and a ^{3}H-activity of 2.32×10^6 dpm $mmol^{-1}$, giving an incorporation of 0.54%. Also isolated was rotenone (30 mg) having a ^{14}C-activity of 2.56×10^5 dpm $mmol^{-1}$, giving an incorporation of 0.84%, and a ^{3}H-activity of 1.10×10^4 dpm $mmol^{-1}$, giving an incorporation of 0.002%.

Degradation of the amorphigenin at a ^{14}C specific activity of 7.08×10^4 dpm $mmol^{-1}$ (taken as 1.00) and a ^{3}H specific activity of 7.03×10^5 dpm $mmol^{-1}$ (taken as 1.00) gave, for the 7′-formaldehyde dimedone, ^{14}C RA: 0.49; ^{3}H RA: 0.01, and for the keto acetate, ^{14}C RA: 0.46, ^{3}H RA: 0.98.

Formation of Amorphigenin and Rotenone by Competitive Administration of [4′-^{14}C]*Rot-2′-enonic Acid* **4** *and* 4′-*Hydroxy*-[4′-^{3}H]*rot-2′-enonic Acid* **18** *to* A. fruticosa *Seedlings* (*Second Experiment*).—[4′-^{14}C]Rot-2′-enonic acid **4**, sufficient to provide 2.89×10^6 dpm, was administered at a specific activity of 1.78×10^9 dpm $mmol^{-1}$ along with 4′-hydroxy-[4′-^{3}H]rot-2′-enonic acid **18**, sufficient to provide 1.40×10^7 dpm at a specific activity of 8.25×10^9 dpm $mmol^{-1}$, to 400 *A. fruticosa* seedlings. Uptake of substrate **4** was 61.7% and that of **18** 46.4%. Amorphigenin (35.7 mg) was isolated having a ^{14}C-activity of 1.95×10^5 dpm $mmol^{-1}$, giving an incorporation of 0.96%, and a ^{3}H-activity of 2.33×10^5 dpm $mmol^{-1}$, giving an incorporation of 0.31%.

Formation of Amorphigenin by Competitive Administration of

5'-Hydroxy-[4'-^{14}C]rot-2'-enonic Acid **19** *and 4'-Hydroxy-4'-^{3}H]rot-2'-enonic Acid* **18** *to* A. fruticosa *Seedlings.*—5'-Hydroxy-[4'-^{14}C]rot-2'-enonic acid **19**, sufficient to provide 2.63×10^{7} dpm at a specific activity of 1.05×10^{10} dpm mmol^{-1}, along with 4'-hydroxy-[4'-^{3}H]rot-2'-enonic acid **18**, sufficient to provide 1.20×10^{8} dpm at a specific activity of 1.00×10^{11} dpm mmol^{-1}, were administered to 450 *A. fruticosa* seedlings. Uptake of substrate **4** was 63.0% and that of **18** 52.2%.

Amorphigenin (22 mg) was isolated having a ^{14}C-activity of 7.01×10^{5} dpm mmol^{-1} giving an incorporation of 0.22%, and a ^{3}H-activity of 2.55×10^{6} dpm mmol^{-1} giving an incorporation of 0.22%.

Formation of Amorphigenin by Competitive Administration of [7-^{14}C]Rotenone **1** *and 4'-Hydroxy-[4'-^{3}H]rotenonic Acid* **18** *to* A. fruticosa *Seedlings.*—[7'-^{14}C]Rotenone **1**, sufficient to provide 3.82×10^{6} dpm at a specific activity of 2.07×10^{9} dpm mmol^{-1}, along with 4'-hydroxy-[4'-^{3}H]rot-2'-enonic acid **18**, sufficient to provide 2.25×10^{7} dpm (specific activity of 8.82×10^{9} dpm mmol^{-1}) were administered together to 450 *A. fruticosa* seedlings, allowing a growth period of 48 h. Uptake of substrate **1** was 64.7% and that of **18** 50.2%. Amorphigenin (36 mg) was isolated having a ^{14}C-activity of 3.53×10^{5} dpm mmol^{-1}, giving an incorporation of 1.26%, and a ^{3}H-activity of 2.69×10^{5} dpm mmol^{-1}, giving an incorporation of 0.21%.

Formation of 4'-Hydroxy-[4'-^{14}C]rot-2'-enonic Acid **18** *from [4'-^{14}C]Rot-2'-enonic Acid* **4** *by* A. fruticosa.—[4'-^{14}C]Rot-2'-enonic acid providing 3.34×10^{6} dpm (specific activity 2.06×10^{9} dpm mmol^{-1}) was administered to 500 seedlings of *A. fruticosa*, with uptake of 40.4%. The 4'-hydroxyrot-2'-enonic acid, formed after 20 h and isolated by addition of 39 mg of 'cold' carrier, had a specific activity of 6.54×10^{4} dpm mmol^{-1}, giving an incorporation of 0.46%.

Formation of 5'-Hydroxy-[4'-^{14}C]rot-2'-enonic Acid **19** *from [4'-^{14}C]Rot-2'-enonic Acid* **4** *by* A. fruticosa.—[4'-^{14}C]Rot-2'-enonic acid providing 3.45×10^{6} dpm (specific activity 2.06×10^{9} dpm mmol^{-1}) was administered to 350 seedlings of *A. fruticosa*, with uptake of 18.6%. The 5'-hydroxyrot-2'-enonic acid, formed after 20 h and isolated by addition of 25.5 mg of 'cold' carrier, had a specific activity of 3.82×10^{4} dpm mmol^{-1}, giving an incorporation of 0.37%.

Reconstructive Syntheses of Labelled Rotenoids as Candidate Precursors in the Biosynthesis of Rotenone and Amorphigenin

(6aS,12aS,5'R)-[7'-^{14}C]- *and* -[8'-^{2}H]-*Rotenone* **1**.—*Rotenone 6'-norketone enol acetate* **26**. Rotenone (8.0 g, 20.3 mmol), THF (20 cm^3), and water (22 cm^3) were stirred with osmium tetraoxide [50 mg, in *tert*-butyl alcohol (5 cm^3)] for 15 min. Sodium metaperiodate (9.0 g, 42 mmol) was added during 90 min. The mixture was stirred overnight, filtered, and H_2S was passed into the filtrate. After filtration, the solution was poured into water, and extracted with chloroform. The chloroform extracts were washed successively with water, saturated aq. sodium hydrogen carbonate, and then brine. Drying ($MgSO_4$) and evaporation gave rotenone 6'-norketone **25** as a gum, which was crystallised from methanol in needles (6.6 g, 82%), m.p. 198–199 °C (lit.,[13] 198–199 °C); ν_{max}/cm^{-1} 1770, 1725 and 1625.

The norketone **25** (500 mg, 1.26 mmol), isopropenyl acetate (7.5 g, 75 mmol) and conc. sulfuric acid (3 drops) were heated on a steam-bath for 3 h, with removal of acetone as it formed. The product was poured into water and worked up as above. The product was chromatographed on flash silica [eluent: chloroform–diethyl ether–hexane (1:2:2)] to give the *enol acetate* **26** (274 mg, 50%), prisms from methanol, m.p. 176 °C; $[\alpha]_D^{18}$ −96.4 ($CHCl_3$) (Found: C, 65.7; H, 5.1%; M$^+$, 438.1351. $C_{24}H_{22}O_8$ requires C, 65.75; H, 5.05%; M, 438.1314); ν_{max}/cm^{-1} 1770, 1712 and 1613; λ_{max}/nm 372, 354, 340i, 316i, 305i, 292, 259i and 253; δ_H 2.30 (3 H, s, C-7'-Me), 2.42 (3 H, s, OAc), 3.31 (2 H, AB part of ABX, 4'-H), 3.86 (3 H, s, OMe), 3.87 (3 H, s, OMe), 4.23 (1 H, dd, *J* 10.4, 6-H^a), 4.53 (1 H, X part of ABX, 5'-H), 5.08 (1 H, dd, *J* 5 and 10, 6-H^b), 5.42 (1 H, dd, *J* 5 and 10, 6a-H), 6.44 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8, 10-H), 6.85 (1 H, d, *J* 8, 11-H) and 7.39 (1 H, s, 1-H).

[7'-^{14}C]*Enol acetate* **27** *from Wittig methylenation.* n-Butyllithium (1.5 mol dm^{-3}, 152 mm^3) was added to a stirred suspension of [^{14}C]methyltriphenylphosphonium iodide (121 mg, containing some unchanged triphenylphosphine) in dry THF (1 cm^3) at 35 °C. After the mixture had been stirred (5 min), a solution of rotenone 6'-norketone enol acetate **26** (100 mg, 0.23 mmol) in THF (1 cm^3) was added under nitrogen by injection and the mixture was stirred (1 h). The product was poured into water and worked up by extraction (chloroform) and chromatography on silica [eluent: hexane–ethyl acetate (65:35)] to give the title radioactive compound (27 mg, 0.062 mmol). A similar reaction with [^{13}C]methyltriphenylphosphonium iodide (9 mg, 0.23 mmol), 90% enriched, gave [7'-^{13}C]rotenone enol acetate, m.p. 162–164 °C (from MeOH); δ_C 112.5 (CH_2, C-7').

On a larger scale unlabelled material was prepared in 56% yield, pale yellow prisms from methanol, m.p. 163–164 °C (lit.,[14] 164–165 °C); $[\alpha]_D^{18}$ −152.1 (*c*, 2.0, benzene) {lit.,[14] $[\alpha]_D^{22}$ −156 (*c* 2.05, benzene)}; δ_H 1.78 (3 H, s, 8'-H_3), 2.40 (3 H, s, OAc), 3.11 (2 H, m, 4'-H_2), 3.84 (6 H, s, 2 × OMe), 4.21 (1 H, d, *J* 10, 6-H^a), 4.49 (1 H, m, 5'-H), 5.08 (3 H, m, 6-H^b and 7'-H_2), 5.38 (1 H, dd, *J* 5 and 10, 6a-H), 6.42 (1 H, d, *J* 8, 10-H), 6.46 (1 H, s, 4-H), 6.83 (1 H, d, *J* 8, 11-H) and 7.39 (1 H, s, 1-H).

Hydrolysis of enol acetate **27** *to* [7'-^{14}C]*rotenone* **1**. [7'-^{14}C]Enol acetate (27 mg, 0.062 mmol) was diluted with unlabelled material (31 mg, 0.071 mmol) and refluxed with conc. hydrochloric acid (0.2 cm^3) in methanol (1.15 cm^3) for 2 h and was then poured into water. Extraction with chloroform, evaporation, and crystallisation from ethanol gave [7'-^{14}C]rotenone, specific activity 66.6 mCi mmol^{-1}. Allowing for dilution the radiochemical yield was 66%. A similar, unlabelled experiment gave rotenone in 83% yield, m.p. 160–162 °C (from MeOH) (lit.,[24] 163 °C); $[\alpha]_D^{24}$ −122.0 (*c* 0.05, $CHCl_3$) (lit.,[24] $[\alpha]_D^{25}$ −127); ν_{max}/cm^{-1} 1670 and 1610. Hydrolysis of the ^{13}C-enol acetate gave [7'-^{13}C]rotenone (79%), m.p. 164 °C (from MeOH); δ_C 113.1 (CH_2, C-7').

(6aS,12aS,5'R)-[8'-^{2}H]*Rotenone.* Rotenone (9.2 g, 23.4 mmol) was stirred with benzeneselenenyl chloride (4.9 g, 25.7 mmol) under nitrogen in anhydrous THF (250 cm^3) for 22 h at room temperature. The product was cooled to 0 °C and hydrogen peroxide (100 vol; 9 cm^3, 2.5 mol equiv.) added to the stirred mixture at 0 °C (10 min), followed by further stirring (30 min) of the mixture at 20 °C. After being poured into aq. sodium hydrogen carbonate and extraction with diethyl ether, the product, dissolved in chloroform, was passed down a short silica column and then purified by HPLC (μPorasil; elution with 20% ethyl acetate in hexane) and crystallisation from ethyl acetate or chloroform–methanol. This gave (6aS,12aS,5'R)-8'-*chlororotenone* **30** (5.1 g, 51%), needles, m.p. 189.5–190 °C; $[\alpha]_D^{30}$ −110 (*c* 0.090, $CHCl_3$) {lit.,[3] 162–164 °C; $[\alpha]_D^{27}$ −97 (*c* 0.08, $CHCl_3$): the reason for the discrepant data is not known} (Found: C, 64.4; H, 4.9%; M$^+$, 428.1036. $C_{23}H_{21}ClO_6$ requires C, 64.4; H, 4.9%; M, 428.1026); ν_{max}(KBr)/cm^{-1} 1670, 1605 and 1510; δ_H 3.27 (2 H, ABX, 4'-H_2), 3.76 and 3.81 (each 3 H, s, OMe), 3.86 (1 H, d, 12a-H), 4.16 (2 H, s, 8'-H_2), 4.15 (1 H, d, *J* 12, 6-H^a), 4.61 (1 H, d, *J* 12, 6-H^b), 5.94 (1 H, m, 6a-H), 5.44 (3 H, m, 5-H and 7'-H_2), 6.47 (1 H, s, 4-H), 6.53 (1 H, d, *J* 9, 10-H), 6.81 (1 H, s, 1-H) and 7.87 (1 H, d, *J* 9, 11-H).

A solution of 8′-chlororotenone **30** (1.1 g, 2.56 mmol) in dry acetone (50 cm^3) was refluxed with sodium iodide (0.96 g, 6.4 mmol) for 2 h under nitrogen. Work-up, with a sodium thiosulfate wash gave, on crystallisation from acetone, (6aS,12aS,5′R)-8′-*iodorotenone* **31** (0.82 g, 61%), needles, m.p. 188–189 °C (decomp.) (Found: C, 53.4; H, 4.05%; M^+, 520.0411. $C_{23}H_{21}IO_6$ requires C, 53.1; H, 4.05%; M, 520.0384).

Sodium cyanoborodeuteride (12 mg, 0.182 mmol) was added to a solution of 8′-iodorotenone **31** (0.1 g, 0.182 mmol) in dry HMPA (1 cm^3) and the mixture was stirred at 65 °C for 2 h under nitrogen. Work-up with diethyl ether in the usual way gave (6a*S*,12a*S*,5′*R*)-[8′-^{2}H]rotenone (43 mg, 60%), plates from chloroform–ethanol, m.p. 162–163 °C; $[\alpha]_D^{24}$ −125.4 (*c* 0.065, $CHCl_3$); $\delta(^2H)$ 1.79 (1 D, br t, *J* 1.83, 8′-D) {lit.,[24] m.p. 163 °C; $[\alpha]_D^{25}$ −127 ($CHCl_3$)}.

(6aS,12aS,5′R)-[7′-^{13}C]- *and* [7′-^{14}C]-*Amorphigenin.*—*Amorphigenin acetate 6′-norketone enol acetate* **34** (X = O). Amorphigenin acetate (298 mg, 0.659 mmol), THF (20 cm^3), and water (8.5 cm^3) were treated with a mixture of osmium tetraoxide (15 mg) in *tert*-butyl alcohol (1.5 cm^3) and, after the mixture had been stirred for 10 min sodium metaperiodate (630 mg, 2.95 mmol) was added. After the mixture was stirred (16 h) and worked up as for compound **25** (above) the amorphigenin analogue **12** (272 mg, 91%) was obtained as needles from chloroform–diethyl ether, m.p. 196–197 °C (lit.,[3] 196 °C).

The keto acetate **12** (314 mg, 0.69 mmol) isopropenyl acetate (9.1 g, 91 mmol), and conc. sulfuric acid (3 drops) were heated at 100 °C for 4.5 h, allowing the acetone thus formed to evaporate. The product was poured into water and extracted with chloroform. Purification by flash chromatography on silica, and elution with benzene–diethyl ether–hexane (6:2:1) gave the *title acetate* **34** (X = O) (172 mg, 50%), m.p. 168–169 °C (from $CHCl_3$–MeOH) (Found: C, 63.2; H, 5.05%; M^+, 496.1366. $C_{26}H_{24}O_{10}$ requires C, 62.9; H, 4.9%; M, 496.1369); λ_{max}(EtOH)/nm 369, 355, 354, 315 and 252; δ_H 2.17 (3 H, s, OAc), 2.42 (3 H, s, enol-OAC), 3.35–3.45 (2 H, AB of ABX, 4′-H_2), 3.86 (3 H, s, OMe), 3.87 (3 H, s, OMe), 4.23 (1 H, dd, $J_{6.6} = J_{6.6a}$ = 10.3, 6-H), 4.54 (1 H, dd, $J_{6.6}$ 10.2, $J_{6.6a}$ 5.2, 6-H), 4.93 (1 H, d, *J* 17.9, 8′-H), 5.06 (1 H, d, *J* 17.9, 8′-H), 5.24 (1 H, X of ABX, 5′-H), 5.44 (1 H, dd, $J_{6.6a}$ 10.4, $J_{6a.6}$ 5.2, 6a-H), 6.44 (1 H, s, 4-H), 6.48 (1 H, d, *J* 8.3, 10-H), 6.85 (1 H, d, *J* 8.3, 11-H) and 7.39 (1 H, s, 1-H); δ_C 20.4 and 21.0 (2 × Me, OCO*Me*), 30.1 (CH_2, C-4′), 55.9 and 56.3 (2 × Me, OMe), 66.5 (CH_2, C-8′), 67.6 (CH_2, C-6), 72.7 (CH, C-6a), 85.6 (CH, C-5′), 101.1 (CH, C-4), 103.2 (CH, C-10), 108.8 (C, C-12), 109.7 (CH, C-1), 111.7 (C, C-12b), 112.5 (C, C-8), 115.1 (C, C-11a), 122.4 (CH, C-11), 134.6 (C, C-12a), 144.2 (C, C-2), 150.2, 150.3 and 150.5 (3 × C, C-3, -4a and -7a), 160.7 (C, C-9), and 167.7 and 170.2 (2 × C, *O*COMe).

6′-Methylenation of the diacetate **34** (X = O)*: labelling with* ^{13}C *and* ^{14}C. A mixture of methyltriphenylphosphonium iodide (39.1 mg, 0.097 mmol) in dry THF (2.5 cm^3) was treated with n-butyllithium (1.46 mol dm^{-3} in hexane; 66 mm^3, 0.097 mmol). The mixture was warmed to 40 °C and stirred under nitrogen (5 min) and a solution of the diacetate **34** (X = O) (48.4 mg, 0.097 mmol) in dry THF (0.5 cm^3) was added in one portion. After being stirred (5 min) at 40 °C the mixture was poured into water and extracted with chloroform and the product was isolated by chromatography on flash silica with ethyl acetate–hexane (3:7) as eluent. The *enol acetate of amorphigenin acetate* **34** (X = CH_2) (26 mg, 54%) formed needles from chloroform–methanol, m.p. 134–136 °C (Found: C, 65.0; H, 5.25%; M^+, 494. $C_{27}H_{26}O_9$ requires C, 65.6; H, 5.25%); δ_H 2.08 (3 H, s, OAc), 2.17 (3 H, s, OAc), 3.08 (1 H, dd, $J_{4′.4′}$ 15.7, $J_{4′.5′}$ 7.9, 4′-H), 3.33 (1 H, dd, $J_{4′.4′}$ 15.7, $J_{4′.5′}$ 9.6, 4′-H), 3.85 (3 H, s, OMe), 3.87 (3 H, s, OMe), 4.23 (1 H, dd, $J_{6.6} = J_{6.6a}$ = 10.3, 6-H), 4.54 (1 H, dd, $J_{6.6}$ 10.2, $J_{6.6a}$ 5.2, 6-H), 4.67 (1 H, d, *J* 13.5, 8′-H), 4.73 (1 H, d, *J* 13.5, 8′-H), 5.27 (1 H, s, 7′-H), 5.31 (1 H, obscured, 5′-H), 5.35 (1 H, s, 7′-H), 5.41 (1 H, dd, $J_{6a.6}$ 10.3, $J_{6a.6}$ 5.2, 6a-H), 6.42 (1 H, d, *J* obscured, 10-H), 6.44 (1 H, s, 4-H), 6.82 (1 H, d, *J* 8.3, 11-H) and 7.39 (1 H, s, 1-H); δ_C 20.9 and 21.0 (2 × Me, OCO*Me*), 32.2 (CH_2, C-4′), 55.9 and 56.3 (2 × Me, OMe), 63.7 (CH_2, C-8′), 67.7 (CH_2, C-6), 72.6 (CH, C-6a), 84.1 (CH, C-5′), 101.0 (CH, C-4), 103.0 (CH, C-10), 109.0 (C, C-12), 109.7 (CH, C-1), 111.4 (C, C-12b), 112.8 (C, C-8), 114.1 (C, C-11a), 114.4 (CH_2, C-7′), 122.1 (CH, C-11), 142.7 (C, C-6′), 144.1 (C, C-2), 150.0, 150.2 and 150.3 (3 × C, C-3, -4a and -7a), 161.6 (C, C-9) and 167.8 and 170.6 (2 × C, *O*COMe).

Use of [^{13}C]methyltriphenylphosphonium iodide (106.4 mg, 0.263 mmol; 90% enrichment) and diacetate **34** (X = O) (125.5 mg, 0.25 mmol) under reaction conditions similar to those above gave the enol acetate of [7′-^{13}C]amorphigenin acetate (82.7 mg, 66%), m.p. 135–136 °C (from $CHCl_3$–EtOH); δ_C 114.1 (CH_2, C-7′).

Similarly, [7′-^{14}C]enol acetate (74.4 mg, 64%) was made from [^{14}C]methyltriphenylphosphonium iodide (99.6 mg, 0.246 mmol; 10.1 mCi $mmol^{-1}$) and the diacetate **34** (X = O) (117 mg, 0.234 mmol). After purification by column chromatography, the whole sample was hydrolysed (see below).

[7′-^{13}C]- *and* [7′-^{14}C]-*Amorphigenin.* Unlabelled enol acetate of amorphigenin acetate (20 mg, 0.04 mmol), methanol (850 mm^3), and hydrochloric acid (150 mm^3; 36% w/v) were heated under reflux for 2 h. Work-up gave amorphigenin **2** (12 mg, 73%), m.p. 196–197 °C, needles from chloroform–methanol (lit.,[19] 196–197 °C); δ_H 1.89 (1 H, br s, OH), 3.06 (1 H, dd, $J_{4′.4′}$ 15.8, $J_{4′.5′}$ 8.6, 4′-H), 3.42 (1 H, dd, $J_{4′.4′}$ 15.8, $J_{4′.5′}$ 9.5, 4′-H), 3.70 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.84 (1 H, d, *J* obscured, 12a-H), 4.17 (1 H, d, *J* obscured, 6-H), 4.26 (2 H, s, 8′-H_2), 4.61 (1 H, dd, $J_{6.6}$ 12, $J_{6.6a}$ 3, 6-H), 4.92 (1 H, m, 6a-H), 5.27 (2 H, br s, 7′-H_2), 5.39 (1 H, X of ABX, 5′-H), 6.45 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.5, 10-H), 6.77 (1 H, s, 1-H) and 7.85 (1 H, d, *J* 8.5, 11-H).

Similar hydrolysis of the ^{13}C-labelled enol acetate (82 mg, 0.166 mmol) gave [7′-^{13}C]amorphigenin **2** (55 mg, 81%), m.p. 193–194 °C (from $CHCl_3$–MeOH); δ_C 112.4 (C-7′).

Similar hydrolysis of the ^{14}C-labelled enol acetate (74.4 mg, 0.150 mmol) gave [7′-^{14}C]-amorphigenin **2** (53.1 mg, 86%; 7.15 mCi $mmol^{-1}$, radiochemical yield 37% from [^{14}C]methyltriphenylphosphonium iodide), m.p. 194–195 °C.

(6aS,12aS)-(E)-[4′-^{14}C]-, -[4′-^{13}C]-, -[4′-^{3}H]- *and* [4′-^{2}H]-*Rot-2′-enonic Acid* **4**.—For reconstructive syntheses from (6a*S*,12a*S*)-4′-bromorot-2′-enonic acid see our earlier work.[12] The selectivity of the direct hydrogenolysis method is indicated by the following experiment. [7′-^{13}C]Rotenone (50.1 mg, 0.127 mmol; 90% enrichment) in dry pyridine (1.7 cm^3) was hydrogenated over 10% barium sulfate (12.8 mg) and gave [4′-^{13}C]rot-2′-enonic acid (23.7 mg, 43%), m.p. 205–206 °C (from EtOH); $\delta(^{13}C)$ [$(CD_3)_2SO$ + Cr(acac)$_3$] 17.5 (C-5′,11% of total) and 25.5 (C-4′, 89% of total).

[4′-^{3}H]*Rot-2′-enonic acid* **4** *from* 4′-*hydroxy*-[4′-^{3}H]*rot-2′-enonic acid* **18**. The unlabelled hydroxy acid (21 mg, 0.43 mmol) and sodium cyanoborohydride (50 mg, 0.79 mmol) were dried under high vacuum overnight and were then added to a mixture of triphenyl phosphite methiodide (330 mg, 0.73 mmol) and HMPA (2 cm^3). The mixture was kept at 65 °C under argon for 2.5 h. After cooling, the product was diluted with saturated aq. ammonium chloride and extracted into diethyl ether. Purification by PLC on silica with dichloromethane–propan-2-ol (99 : 1) gave rot-2′-enonic acid (10.5 mg, 60%) having an NMR spectrum identical with that of an authentic specimen.

4′-Hydroxy-[4′-^{3}H]rot-2′-enonic acid (20 mg, 4.7 × 10^8 dpm mg^{-1}) was then treated as above to give, after PLC, [4′-^{3}H]rot-2′-enonic acid (55 mg; 339 μCi). ^{3}H NMR spectroscopy showed the presence of only one signal, at δ_T 1.69.

(6aS,12aS,5'R)-[8'-^{14}C]*Amorphigenin.—Direct conversion of* (6aS,12aS,5'R)-*Rotenone* **1** *into* (6aS,12aS,5'R)-*Amorphigenin* **2**. Potassium phthalimide (300 mg), benzene selenenyl chloride (287 mg), and dry (CaH_2) and degassed hexane (2 cm^3) were vigorously stirred together under argon at 25 °C for 2 h. Dry (CaH_2) dichloromethane (10 cm^3) was added and the solution was concentrated under reduced pressure to 2 cm^3, when dry hexane (8 cm^3) was added. The crystals were filtered off and washed with dry hexane to give *N*-phenylselenophthalimide (N-PSP) **35** (392 mg).

A solution of the latter (111 mg, 0.37 mmol) in dichloromethane (5 cm^3) containing water (17 mm^3, 2.5 mol equiv.) and camphorsulfonic acid (CSA) (8.5 mg, 0.037 mmol) was added to rotenone (145 mg, 0.37 mmol) and the mixture was stirred for 65 h at ambient temperature. The solvent was removed under reduced pressure and pyridine (20 mm^3 mg^{-1} rotenone used) was added. After the mixture had been cooled to 0 °C hydrogen peroxide (30%; 0.5 cm^3) was added, and after being kept at 0 °C for 30 min the solution was allowed to warm to 20 °C and stirred for another 1.5 h. The mixture was poured into saturated aq. sodium hydrogen carbonate and extracted with chloroform. After washing, drying, and evaporation of the extracts, the product was chromatographed on silica and eluted with ethyl acetate–hexane (1:3) to give (6a*S*,12a*S*,5'*R*)-amorphigenin **2** (54 mg, 36%), needles from chloroform–ethanol, m.p. and mixed m.p. with natural material 196–197 °C, $[\alpha]_D^{24}$ −123.6 (*c* 0.17, $CHCl_3$) {lit.,[19] m.p. 196–197 °C; $[\alpha]_D^{20}$ −125.6 (*c* 2.04, $CHCl_3$)} (Found: C, 65.65; H, 5.9%; M^+, 410.1320. Calc. for $C_{23}H_{22}O_7$·CH_3OH. C, 65.15; H, 5.9%; M, 410.1357).

By using [7'-^{14}C]rotenone, [8'-^{14}C]amorphigenin was prepared by the above procedure and the distribution of ^{14}C label, as between C-8' and C-7', was investigated by our previously described degradation method. Formaldehyde dimedone arising from C-7' contained 90.8% of the original radioactivity, whilst that from C-8' contained 7.5%.

Conversion of (6aS,12aS,5'R)-*Rotenone into* (6aS,12aS,5'R)-*amorphigenin* via *the enol acetate procedure*. Rotenone (2.1 g), isopropenyl acetate (15 cm^3), and conc. sulfuric acid (10 drops) were heated at 100 °C for 4 h with removal of acetone as it was produced. Work-up by chromatography on silica [eluent hexane–diethyl ether–chloroform (3:3:1)] gave rotenone enol acetate **27** (11.5 g, 50%), m.p. 164 °C (from EtOH) (lit.,[14] 164–165 °C).

The enol acetate (102 mg, 0.24 mmol) was added to a stirred solution of N-PSP (71 mg, 0.24 mmol) in dichloromethane containing CSA (6 mg) and water (10 mm^3). After 3 days in the absence of light the solvent was removed to leave a yellow gum, which was dissolved in THF (5 cm^3). Basic alumina (200 mg) and hydrogen peroxide (30%; 0.5 cm^3) were added and the mixture was stirred at 50 °C (4 h) and then poured into water and extracted with chloroform. Work-up, and chromatography on silica [eluent ethyl acetate–hexane (1:3)], gave amorphigenin enol acetate (50%), which was used directly in the next step; δ_H 7.41 (1 H, s, 1-H), 6.91 (1 H, d, *J* 9, 10-H), 6.48 (1 H, s, 4-H), 6.43 (1 H, d, *J* 9, 9-H), 5.45 (2 H, m, 6a- and 5'-H), 5.27 (2 H, br, 7'-H_2), 4.40 (2 H, m, 6-H_2), 3.87 (6 H, s, 2 × OMe), 3.20 (2 H, m, 4'-H_2) and 2.41 (3 H, s, OAc).

Amorphigenin enol acetate (50 mg, 0.11 mmol), conc. hydrochloric acid (350 mm^3), and methanol (2.5 cm^3) were refluxed (2 h) and then worked up. Purification by PLC [silica; solvent ethyl acetate–hexane (1:1)] gave amorphigenin **2** (30 mg, 67%), which was crystallised from chloroform–methanol as the monomethanol solvate, m.p. 192–193 °C (lit.,[19] 196–197 °C). The ^{1}H NMR spectrum was identical with that of authentic natural material.

(6aS,12aS)-(Z)-5'-*Hydroxy*-[4'-^{13}C]- *and* -[4'-^{14}C]-*rot*-2'-*enonic Acid*, (E)-4'-*Hydroxy*-[5'-^{13}C]-, -[5'-^{14}C]-, [4'-^{2}H]- *and* -[4'-^{3}H]-*rot*-2'-*enonic Acid.—Hydrogenolysis of amorphigenin and its* [^{13}C]- *and* [^{14}C]-*labelled variants*. Amorphigenin (44.2 mg, 0.108 mmol) in pyridine (1.7 cm^3) was hydrogenated in a Brown microhydrogenator over a 10% palladium-on-barium sulfate catalyst until one mol of hydrogen had been absorbed. The reaction mixture was filtered and extracted with chloroform. The extracts were washed free from pyridine by using hydrochloric acid followed by water. Drying, evaporation, and purification by PLC on silica, with chloroform–propan-2-ol (20:1) gave, in order of elution, three compounds. First, (6aS,12aS)-5'-*hydroxyrot*-2'-*enonic acid* **19** (10.2 mg, 23%), m.p. 188–189 °C (from MeOH) (Found: C, 65.2; H, 6.05%; M^+, 412.1495. $C_{23}H_{24}O_7$·CH_3OH requires C, 64.85; H, 6.35%; M, 412.1522); ν_{max}/cm^{-1} 3430 (OH) and 1652 (C=O); $\delta_H(CDCl_3)$ 1.74 (1 H, s, 4'-H_3), 3.47 (2 H, AB of ABX, 1'-H), 3.77 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.83 (1 H, d, *J* 4, 12a-H), 4.19 (1 H, d, *J* 12, 6-H), 4.25 (1 H, d, *J* 11.8, 5'-H), 4.40 (1 H, d, *J* 11.8, 5'-H), 4.65 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3, 6-H), 4.93 (1 H, m, 6a-H), 5.32 (1 H, X of ABX, 2'-H), 6.44 (1 H, s, 4-H), 6.51 (1 H, d, *J* 8.7, 10-H), 6.79 (1 H, s, 1-H) and 7.74 (1 H, d, *J* 8.7, 11-H); $\delta_C[(CD_3)_2SO]$ 21.1 (CH_3 and CH_2, C-1' and -4'), 43.2 (CH, C-12a), 55.5 and 56.1 (2 × Me, OMe), 59.45 and 65.8 (2 × CH_2, C-6 and -5'), 71.45 (CH, C-6a), 101.3 (CH, C-4), 105.4 (C, C-12b) 110.0 (CH, C-1), 111.1 (CH, C-10), 111.6 (C, C-8), 114.2 (C, C-11a), 122.9 (CH, C-2'), 126.1 (CH, C-11), 135.3 (C, C-3'), 143.1 (C, C-2), 147.7 (C, C-4a), 149.3 (C, C-3), 159.8 (C, C-9), 162.2 (C, C-7a) and 189.2 (C, C-12).

This was followed by (6aS,12aS)-5'-*hydroxyrot*-3'-*enonic acid* **43** (24.3 mg, 55%), m.p. 182–184 °C (from MeOH) (Found: M^+, 412.1486. $C_{23}H_{24}O_7$ requires M, 412.1552; ν_{max}/cm^{-1} 3345 (OH) and 1650 (C=O); $\delta_H(CDCl_3)$ 2.30 (2 H, m, 2'-H_2), 2.82 (2 H, m, 1'-H_2), 3.75 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.75–3.79 (1 H, obscured, 12a-H), 4.16 (1 H, d, *J* obscured, 6-H), 4.21 (1 H, s, 4'-H), 4.53 (1 H, s, 4'-H), 4.62 (1 H, dd, *J* obscured, 6-H), 4.82 (1 H, m, 6a-H), 6.44 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.7, 10-H), 6.77 (1 H, s, 1-H) and 7.72 (1 H, d, *J* 8.7, 11-H); $\delta_C[(CD_3)_2SO]$ 20.9 and 31.6 (2 × CH_2, C-1' and -2'), 43.2 (CH, C-12a), 55.5 and 56.1 (2 × Me, OMe), 63.6 and 65.9 (2 × CH_2, C-6 and -5'), 71.5 (CH, C-6a), 101.4 (CH, C-4), 105.5 (C, C-12b), 107.7 (CH_2, C-4'), 110.0 (CH, C-1), 111.2 (CH, C-10), 111.6 (C, C-8), 114.9 (C, C-11a), 126.1 (CH, C-11), 143.1 (C, C-2), 147.7 (C, C-4a), 149.3 (C, C-3), 149.5 (C, C-3'), 160.2 (C, C-9), 162.4 (C, C-7a) and 189.3 (C, C-12).

The final compound in elution order was (6a*S*,12a*S*)-4'-hydroxyrot-2'-enonic acid **18** (11.1 mg, 25%), m.p. 211–212 °C (from MeOH) (see later); ν_{max}/cm^{-1} 3530 (OH), 3160 (OH) and 1660 (C=O); $\delta_H[(CD_3)_2SO]$ 1.69 (3 H, s, 5'-H_3), 3.25 (2 H, AB of ABX, 1'-H_2), 3.58 (3 H, s, OMe), 3.69 (3 H, s, OMe), 3.70 (2 H, d, *J* obscured, 4'-H_2), 3.85 (1 H, d, *J* 4, 12a-H), 4.21 (1 H, d, *J* 12, 6-H), 4.50 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3, 6-H), 5.01 (1 H, m, 6a-H), 5.34 (1 H, X of ABX, 2'-H), 6.49 (1 H, s, 4-H), 6.54 (1 H, d, *J* 8.7, 10-H), 6.64 (1 H, s, 1-H) and 7.76 (1 H d, *J* 8.7, 11-H).

[7'-^{13}C]Amorphigenin (29.4 mg, 0.072 mmol; 44% enrichment) was similarly hydrogenolysed to give 5'-hydroxy-[4'-^{13}C]rot-2'-enonic acid **19** (7.4 mg, 25%), m.p. 189–190 °C (from MeOH); δ_C(20 MHz; $CDCl_3$–CD_3OD) 21.6 (CH_3, C-4'); and 4'-hydroxy-[5'-^{13}C]rot-2'-enonic acid **16** (7.5 mg, 25%), m.p. 210–212 °C (from MeOH); δ_C(20 MHz; $CDCl_3$–CD_3OD) 13.5 (CH_3, C-5').

[7'-^{14}C]Amorphigenin (32.8 mg, 0.08 mmol; 4.73 mCi $mmol^{-1}$) was similarly hydrogenolysed and purified to give 5'-hydroxy-[4'-^{14}C]rot-2'-enonic acid **19** (7.5 mg, 23%) and 4'-hydroxy-[5'-^{14}C]rot-2'-enonic acid **16** (7.0 mg, 21%). The ^{1}H NMR spectra were identical with those of the unlabelled specimens above, and the products had the same specific activities as their radioactive precursor.

(6aS,12aS)-4'-*Hydroxyrot*-2'-*enonic acid* **18** *from rotenone*. A solution of rotenone (1.00 g, 2.54 mmol) in dry dichloromethane

(6 cm^3) was added to boron tribromide (2.54 cm^3; 1 mol dm^{-3} solution) in dry dichloromethane (4 cm^3) at −5 to −10 °C. The mixture was stirred (2 min), evaporated to dryness, and slowly treated with ice-cold methanol. Storage at 0 °C produced crystals, which on recrystallisation by gradual addition of methanol to a concentrated dichloromethane solution at 0 °C gave (6a*S*,12a*S*)-4′-bromorot-2′-enonic acid **39** (0.69 g, 57%), needles, m.p. 151–152.5 °C; $[\alpha]_D^{26}$ +26.6 (*c* 0.18, $CHCl_3$) {lit.,[12] m.p. 152–154 °C; $[\alpha]_D^{20}$ +27.2 (*c* 0.72, $CHCl_3$)}; ν_{max}/cm^{-1} 1655.

The latter product (250 mg, 0.53 mmol) was stirred (24 h) with silver oxide (125 mg, 0.53 mmol) in dry acetone (10 cm^3) at 20 °C in the dark. The mixture was filtered through Celite, diluted with chloroform, washed successively with water and brine, and evaporated. Chromatography on silica, with chloroform containing 2% methanol as eluent, gave (6aS,12aS)-*4′-hydroxyrot-2′-enonic acid* (129 mg, 52%), needles from methanol, m.p. 212–213 °C, as the mono-methanol solvate (Found: C, 64.8; H, 6.35%; M^+, 412.1493. $C_{23}H_{24}O_7{\cdot}CH_3OH$ requires C, 64.85; H, 6.35%; M, 412.1522); δ_H 1.80 (3 H, s, 5′-H_3), 3.00 (2 H, br s, OH), 3.36 (2 H, br d, *J* 7.4, 1′-H_2), 3.64 and 3.75 (each 3 H, s, OMe), 3.85 (1 H, obscured, 12a-H), 3.86 (2 H, s, 4′-H_2), 4.46 (2 H, ABX, J_1 3.1, J_2 12.1, 6-H_2), 5.05 (1 H, m, 6a-H), 5.46 (1 H, br t, 2′-H), 6.45 (1 H, s, 4-H), 6.59 (1 H, d, *J* 8.7, 10-H), 6.75 (1 H, s, 1-H) and 7.63 (1 H, d, *J* 8.7, 11-H).

The 4′-hydroxy compound **18** could also be made by stirring of 4′-bromorot-2′-enonic acid (1 g, 2.11 mmol) in acetone (75 cm^3)–water (4 cm^3) at 60–65 °C for 20 h. Work-up and chromatography on silica, with ethyl acetate–n-hexane (1:1) as eluent, gave the title compound (490 mg, 56%), m.p. 213–214 °C (from MeOH). The ^1H NMR spectrum was identical with that of the sample above.

(6aS,12aS)-*4′-Methoxy*-9-O-*methylrot-2′-enonic acid* **48**. A solution of 4′-hydroxyrot-2′-enonic acid (0.20 g, 0.49 mmol) in neutral chloroform (25 cm^3) containing iodomethane (1 cm^3) and silver oxide (4 g) was stirred at 55–60 °C for 4.5 h, further iodomethane (1 cm^3) being added after 2.5 h. The mixture was filtered through Celite, evaporated, and chromatographed on silica, with chloroform as eluent, to give the title compound (140 mg, 67%) as an oil. Its purity was verified by HPLC on μ-Porasil with chloroform–propan-2-ol (100:1) as eluent and it had $[\alpha]_D^{26}$ +30.8 (*c* 0.14, $CHCl_3$). The reference specimen was made by treatment of (6a*S*,12a*S*)-4′-bromorot-2′-enonic acid (3.0 g) in diethyl ether–methanol (1:1; 30 cm^3) with excess of diazomethane in diethyl ether. After the mixture had been stirred (24 h; 18 °C), benzene (80 cm^3), dil. acetic acid (10 cm^3), and water (100 cm^3) were added. Washing, drying, and evaporation gave a crude product which showed two major spots on TLC (chloroform containing 1% of propan-2-ol), R_f 0.7 and 0.8. The two major products were separated by preparative HPLC [silica; eluent chloroform–propan-2-ol (1:1)] and gave (6a*S*,12a*S*)-4′-bromo-9-*O*-methylrot-2′-enonic acid, m.p. 135–137 °C; $[\alpha]_D^{22.5}$ +31 (*c* 0.3, $CHCl_3$){lit.,[12] m.p. 135–137 °C; $[\alpha]_D^{22.5}$ +31 (*c* 0.3, $CHCl_3$)} and (6a*S*,12a*S*)-4′-methoxy-9-*O*-methylrot-2′-enonic acid **48**, $[\alpha]_D^{24}$ +30.4 (*c*, 0.054, $CHCl_3$). Both specimens were HPLC pure.

(6aS,12aS)-*4′-Acetoxyrot-2′-enonic acid*. A solution of 4′-bromorot-2′-enonic acid (200 mg) in glacial acetic acid (7 cm^3) was stirred under nitrogen with silver acetate (70 mg) for 2.5 h in the dark. Work-up by chromatography as above gave (6aS,12aS)-*4′-acetoxyrot-2′-enonic acid* (120 mg, 60%), needles from methanol, m.p. 158.5–159.5 °C; $[\alpha]_D^{30}$ +10.76 (*c* 0.16, $CHCl_3$) (Found: C, 66.0; H, 5.85%; M^+, 454.1632. $C_{25}H_{26}O_8$ requires C, 66.05; H, 5.75%; M, 454.1682); ν_{max}(KBr)/cm^{-1} 1740 and 1655; λ_{max}/nm 317 (log ε 3.86) and 290 (4.07); δ_H 1.82 (3 H, s, 5′-H_3), 2.06 (3 H, s, OAc), 3.41 (2 H, m, 1′-H_2), 3.75 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.84 (1 H, d, *J* 4.1, 12a-H), 4.40 (2 H, m, J_1 12.0, J_2 3.2, 6-H_2), 4.43 (2 H, s, 4′-H_2), 4.92 (1 H, m, 6a-H), 5.53 (1 H, br t, 2′-H), 6.44 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.7, 10-H), 6.76 (1 H, s, 1-H), 6.76 (1 H, s, OH) and 7.72 (1 H, d, *J* 8.7, 11-H).

The acetoxy compound (100 mg, 0.22 mmol) was hydrolysed by treatment with potassium carbonate in methanol–water at 20 °C (2.5 h) and gave a specimen of 4′-hydroxyrot-2′-enonic acid, m.p. 190–193 °C (from MeOH). Converted as above into the tetrakismethyl ether **48**, the latter specimen had $[\alpha]_D^{24}$ +4.2 (*c* 0.050, $CHCl_3$).

Oxidation of 4′-hydroxyrot-2′-enonic acid **18** *to 4′-Oxorot-2′-enonic acid* **46**. A solution of the hydroxy acid (501 mg, 1.21 mmol) in dry DMF (300 mm^3) was added to a solution of PDC (680 mg, 1.83 mmol) in dry DMF (900 mm^3) at 0 °C. After being stirred at 0 °C for 4.5 h, the product was poured into ethyl acetate (15 cm^3) and filtered through Kieselguhr. After work-up the product was evaporated at 4 mmHg and the residue was chromatographed on flash silica and eluted with ethyl acetate–hexane (2:3) to give (6aS,12aS)-*4′-oxorot-2′-enonic acid* (324 mg, 65%), m.p. 162–163 °C (from $CHCl_3$–MeOH) (Found: C, 67.0; H, 5.65; M^+, 410.1355. $C_{23}H_{22}O_7$ requires C, 67.3; H, 5.4%; M, 410,1366); δ_H 1.88 (3 H, s, 5′-H_3), 3.65–3.73 (2 H, AB of ABX, 1′-H_2), 3.72 (3 H, s, OMe), 3.78 (3 H, s, OMe), 3.78–3.87 (1 H, obscured, 12a-H), 4.16 (1 H, d, *J* 12, 6-H), 4.61 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3, 6-H), 4.93 (1 H, m, 6a-H), 6.42 (1 H, s, 4-H), 6.53 (1 H, d, *J* 9, 10-H), 6.48–6.58 (1 H, obscured X of ABX, 2′-H), 6.73 (1 H, s, 1-H), 7.39 (1 H, br s, OH), 7.74 (1 H, d, *J* 9, 11-H) and 9.30 (1 H, s, CHO).

4′-Hydroxy-[4′-^{2}H]- *and* [4′-^{3}H]-*rot-2′-enonic acid* **47** *from unlabelled rot-2′-enonic acid*. Rot-2′-enonic acid (25 mg, 0.06 mmol) was refluxed (16 h) with selenium dioxide (15 mg, 0.13 mmol) in ethanol (1.9 cm^3). The solvent was evaporated off and the residue was taken up in chloroform, washed, and the solution was evaporated to give a yellow oil, which was purified by PLC, with ethyl acetate–hexane (2:3). Crystallisation from chloroform–methanol gave 4′-oxorot-2′-enonic acid **46** (17 mg, 68%), m.p. 161–162 °C; *m/z* 410 (26%, M^+). The ^1H NMR spectrum was identical with that above.

A mixture of sodium borohydride (4.6 mg, 0.122 mmol) in dry diglyme (0.46 cm^3) was added to a solution of 4′-oxorot-2′-enonic acid (50 mg, 0.122 mmol) in diglyme (0.5 cm^3), and after the mixture had been stirred (10 min) it was worked up by being poured into saturated aq. ammonium chloride and extracted with chloroform. PLC with chloroform–propan-2-ol (20:1) gave 4′-hydroxyrot-2′-enonic acid **18** (39.1 mg, 78%), m.p. 211–212 °C (from MeOH); δ_H[$(CD_3)_2SO$] 1.70 (3 H, s, 5′-H_3), 3.25 (2 H, AB of ABX, 1′-H_2), 3.58 (3 H, s, OMe), 3.69 (3 H, s, OMe), 3.70 (2 H, d, *J* obscured, 4′-H_2), 3.85 (1 H, d, *J* 4, 12a-H), 4.21 (1 H, d, *J* 12, 6-H), 4.50 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3, 6-H), 5.01 (1 H, m, 6a-H), 5.34 (1 H, X of ABX, 2′-H), 6.49 (1 H, s, 4-H), 6.55 (1 H, d, *J* 9, 10-H), 6.64 (1 H, s, 1-H) and 7.56 (1 H, d, *J* 9, 11-H); δ_C[$(CD_3)_2SO$] 13.4 (Me, C-5′), 21.0 (CH_2, C-1′), 43.2 (CH, C-12a), 55.5 (Me, OMe), 56.1 (Me, OMe), 65.9 and 66.3 (2 × CH_2, C-4′ and -6), 71.5 (CH, C-6a), 101.3 (CH, C-4), 105.4 (C, C-12b), 110.0 (CH, C-1), 111.1 (CH, C-10), 111.6 (C, C-8), 114.4 (C, C-11a), 120.8 (CH, C-2′), 126.0 (CH, C-11), 135.3 (C, C-3′), 143.2 (C, C-2), 147.7 (C, C-4a), 149.3 (C, C-3), 159.8 (C, C-9), 162.4 (C, C-7a) and 189.17 (C, C-12); *m/z* 412 (24%, M^+).

A similar experiment using 4′-oxorot-2′-enonic acid **46** (43 mg, 0.105 mmol) and sodium boro[^{2}H]hydride gave 4′-hydroxy[4′-^{2}H]rot-2′-enonic acid (33 mg, 76%), m.p. 210–211 °C; *m/z* 413 (13%, M^+).

Similarly, 4′-oxorot-2′-enonic acid (50 mg, 0.122 mmol) in dry diglyme (400 mm^3), when treated with sodium borohydride (0.1 mg) followed by sodium boro[^{3}H]hydride (0.32 mg, 100 mCi) in diglyme (250 mm^3), gave 4′-hydroxy[4′-^{3}H]rot-2′-enonic acid **47** (43.4 mg, 86%). Unlabelled rot-2′-enonic acid (39.6 mg) was added and the whole was crystallised to constant count from methanol. This gave 4′-hydroxy[4′-^{3}H]rot-2′-

enonic acid (39.6 mg), m.p. 212–213 °C, with a specific activity of 88.6 mCi $mmol^{-1}$; $\delta_T[(CD_3)_2SO]$ 3.70 (^3H, s, 4′-H^a) and 3.72 (^3H, s, 4′-H^b).

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