# Biosynthesis of Rotenoids by *Amorpha fruticosa*: Sequence, Specificity, and Stereochemistry in the Development of the Hemiterpenoid Segment

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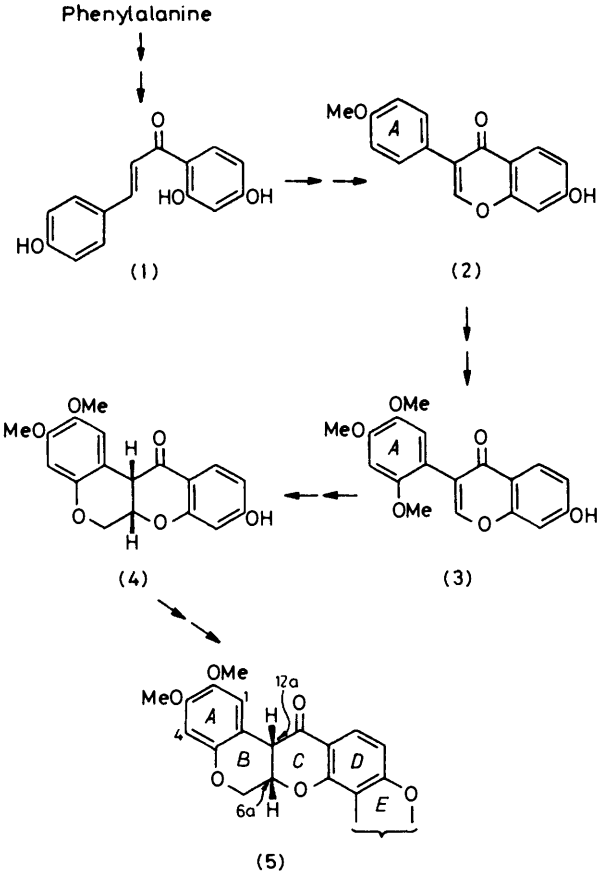
By isolation and radiochemical methods rot-2'-enonic acid, dalpanol, rotenone, and amorphigenin have been identified in *Amorpha fruticosa* seedlings, and ordered in biosynthetic sequence. Its specific activity shows that 12aβ-hydroxyamorphigenin has origins other than direct 12aβ-hydroxylation of amorphigenin: its occurrence and labelling establish it as a true natural product.

Except for isopentenyl alcohol, the potential hemiterpene precursors mevalonic acid, 3-hydroxy-3-methylglutaric acid, and dimethylallyl alcohol are poor precursors for amorphigenin. By employing the already prenylated (*E*)-[4'-$^{14}$C]rot-2'-enonic acid, it is shown that the 4'-C of this compound becomes 7'-C of rotenone. By assuming normal rear-side attack on an intermediate epoxide, and utilising the known absolute configurations at position 5' of dalpanol and rotenone, a stereochemical sequence can be written. (*E*)-4'-Labelled rot-2'-enonic acid leads to a (2'*S*,3'*S*)-epoxide, which on intramolecular attack by phenolate anion would give (5'*R*,6'*S*)-dalpanol, dehydration to rotenone then involving the labelled (*pro*-*S*)-7'-methyl group of dalpanol.

Neither (6'*R*)- nor (6'*S*)-amorphigenol is a precursor of amorphigenin. Administration of [7'-$^{14}$C]rotenone to *A. fruticosa* seedlings has led, in three experiments designed to avoid inadvertent chemical scrambling of the allylic label, to amorphigenin having even label distribution between C-7' and C-8'. Possible interpretations are considered.

USING a germinating seed system from *Amorpha fruticosa*, we have clarified a good deal of the biosynthetic pathway leading to the essential *A*/*B*/*C*/*D* nucleus of the major rotenoid of this plant, amorphigenin (5e).[1-3] In complementary experiments we have used rooted cuttings of *Derris elliptica* in which the major natural product is the 8'-deoxy-relative, rotenone (5d).[2,3] 2',4',4-Trihydroxychalcone (1) is an early intermediate, and an aryl-migration/*O*-methylation sequence leads on to formononetin (2): 2',5'-oxygenation of ring *A* then takes place leading to 7-hydroxy-2',4',5'-trimethoxyisoflavone (3), a key intermediate (Scheme 1). This isoflavone is converted into 9-demethylmunduserone (4), a reaction which involves functionalisation of the 2'-*O*-methyl of (3). In this way the characteristic *A*/*B*/*C*/*D* ring system is built up, and in some plants, *e.g. Mundulea sericea*, 9-demethylmunderone is found trapped as its *O*-methyl ether, munduserone.[4] In most rotenoid-bearing plants however, prenylation occurs and our work on the *Amorpha* system shows this to be effected at a late stage, after completion of the tetracyclic *A*/*B*/*C*/*D* system.[1] Oxidation and involvements with the unblocked 9-hydroxy-group lead to various ring-*E* systems characteristic of the known natural rotenoids. Just as 9-demethylmunduserone stands at the head of one series of rotenoids, others carrying a 2,3-methylenedioxy- in place of the 2,3-dimethoxy-group, or an additional 11-hydroxy-group, are envisaged as standing at the heads of other series.[1]

Our earlier demonstration that [6-$^{3}$H]rot-2'-enonic acid (5a) and [6-$^{3}$H]rotenone are satisfactorily incorporated into amorphigenin by *A. fruticosa* seedlings,[1] suggests that the first product of prenylation of 9-demethylmunduserone (4) is rot-2'-enonic acid. Oxidative cyclisation would then lead to dalpanol (5c),[5] a reaction readily modelled *in vitro* in terms of peroxy-acid–sodium carbonate treatment of rot-2'-enonic acid. Subsequent hydration gives rotenone (5d), with amorphigenin (5e) [6] being a product of further oxidation. The occurrence of rotenoids having a furan ring *E* [*e.g.* elliptone (5q), from *Derris elliptica*] [7] can be ascribed to 4'-hydroxylation of dalpanol followed by fragmentation with loss of acetone, as has been postulated in other systems.[8]




SCHEME 1 Main stages in rotenoid biosynthesis

In this paper we begin to probe and develop these indications of the way in which ring *E* elaboration occurs in rotenoids: in particular we have begun to look at stereochemical aspects which have not been examined hitherto in these or allied natural systems. Three facets are treated here: (i) demonstration of the actual presence of presumptive prenylated intermediates in the amorphigenin formation sequence; (ii) examination of the stereochemistry of the transformation of rot-2′-enonic acid into rotenone, which includes the stereochemistry of dalpanol formation and its dehydration to rotenone; and (iii) examination of the regiospecificity of hydroxylation of rotenone to give amorphigenin.

(i) *The Prenylated Intermediates of* A. fruticosa.—Ungerminated seeds of *A. fruticosa* contain amorphigenin (5e) and its vicianoside amorphin,[6] amorphigenol (5f) into dalpanol, indicating its involvement in the metabolic scheme.

The presence of dalpanol (5c) as a metabolite of *A. fruticosa* received added support from an examination of methanolic extracts of the seeds. After removal of amorphigenin, the concentrated extract deposited a mixture of glycosides which on separation by preparative layer chromatography afforded the vicianoside and glucopyranoside of 6a,12a-didehydrodalpanol. After hydrolysis, the didehydrorotenoid aglycone itself was isolated, verifying that the dalpanol type *E* ring is formed by this plant. Indeed the origins of the didehydrodalpanol (6c) are likely to be dalpanol or its 12a-hydroxy-derivative. Didehydrorotenoids are often claimed as new natural products without adequate proof: oxidation of the *B*/*C* rings of rotenoids to the 6a,12a-didehydro-

TABLE 1

Incorporation of [2-$^{14}$C]phenylalanine into prenylated rotenoids by *Amorpha fruticosa* seedlings [a]

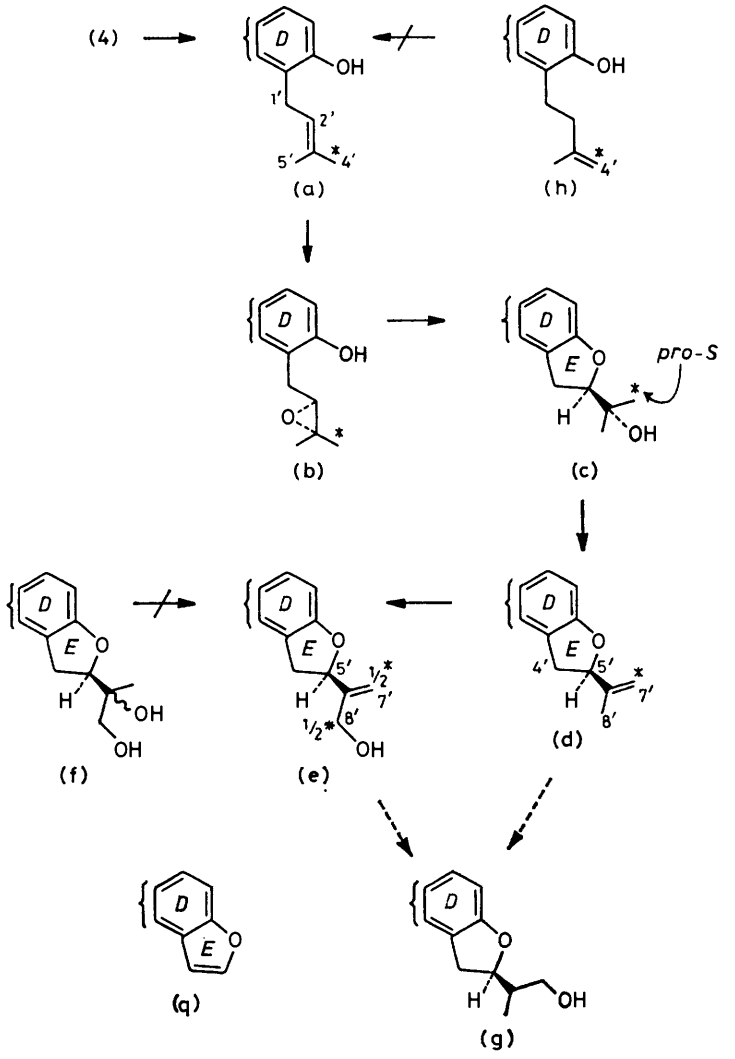
| Rotenoid isolated | | Administration time (h) | Total activity (disint. min$^{-1}$ × $10^{-2}$) | Incorporation (% × $10^{-2}$) |
|---|---|---|---|---|
| Amorphigenin (5e) | 1.51 mg | 6 | 171.1 | 6 |
| | 1.91 mg | 11 | 206.4 | 7 |
| | 3.47 mg | 24 | 833.8 | 30 |
| | 3.60 mg | 48 | 1 336.9 | 48 |
| | 6.25 mg | 48 | 371.0 | 52 [b] |
| | 6.89 mg | 48 | 172.8 | 23 [b] |
| Rotenone (5d) | | 11 | 113.3 | 4 |
| | | 24 | 28.8 | 1 |
| | | 48 | 89.2 | 3 |
| | | 48 | 260.2 | 9 |
| | | 48 | 152.8 | 2 [b] |
| Rot-2′-enonic acid (5a) | | 6 | 61.1 | 2 |
| | | 11 | 74.5 | 3 |
| | | 24 | <0.3 | 0 |
| | | 48 | <0.9 | 0 |
| Dalpanol (5c) | | 48 | 132.7 | 1.2 [c] |

[a] Except where indicated otherwise, 12.5 μCi of [2$^{14}$C]phenylalanine per 60 seedlings for each experiment. [b] 33 μCi of [2$^{14}$C]-phenylalanine per 100 seedlings. [c] 50 μCi of [2$^{14}$C]phenylalanine per 100 seedlings.

and its vicianoside and β-D-glucoside,[9] and dihydroamorphigenin (5g), [10] as well as 6a,12a-didehydrotoxicarol,[11] and 5,7-dihydroxy-6-geranylflavanone.[12] To investigate metabolically active rotenoids on the pathway to amorphigenin, 7—10-day-old (from initiation of germination) seedlings of *A. fruticosa* were used. Single doses of [2-$^{14}$C]phénylalanine were applied to batches of seedlings grown in light to maximise the amorphigenin content, and harvesting was carried out after various growth periods. Rotenoids (5a, c, d, and e) were isolated after addition of unlabelled carrier material, and each was crystallised to constant activity. The results are summarised in Table 1, which shows formation of all four rotenoids. Incorporations into amorphigenin increased steadily over 48 h, showing that the rate of synthesis exceeds the rate of removal: we have, however, found that there is turnover of amorphigenin, although its metabolic fate is not known. After 6 h, incorporation into rotenone was found, though it did not follow a simple time function. Incorporations into rot-2′-enonic acid were low and were detectable only after shorter administration times (6 and 11 h), indicating rapid turnover of the intermediate. Labelling was transferred level can occur directly or *via* a 12a-hydroxy-intermediate [13] and didehydro-rotenoids frequently form during chemical manipulations in air. In addition, 12a-hydroxy-rotenoids are formed from rotenoids *in vivo* (see later) and are dehydrated comparatively readily under *in vitro* conditions.

The rotenoids of *A. fruticosa* (5a—e) are most readily interconnected by the transformations of Scheme 2 in which the epoxide (5b) links rot-2′-enonic acid with the dihydrofuranoid *E*-ring forms.[1] Synthetic work in our laboratory indicates that this epoxide cyclises rapidly under weakly basic conditions to give (5c), and we have not yet succeeded in demonstrating its presence in the seedlings. Since only (5′*R*)-stereoisomers of rotenone [14,15] and its relatives occur naturally, one may conclude that the enzyme-mediated epoxidation of (5a) is stereospecific. We note that although the transformation (5b) ⟶ (5c) is probably enzyme-mediated, (5c) would form as the product of a non-enzymic process, assuming normal rear-side attack by phenolate anion on the epoxide.

(ii) *The Stereochemistry of the Rot-2′-enonic* ⟶ *Rotenone Transformation.*—Certain information on the inter-



SCHEME 2 Prenyl transformations in rotenoid biosynthesis in *Amorpha fruticosa*

relationships amongst (5a—e) could be obtained by applying labelled mevalonates to the problem. Unfortunately incorporations of mevalonate into rotenoids,

TABLE 2

Incorporation of potential terpenoid precursors into amorphigenin (*A. fruticosa*) or rotenone (*D. elliptica*)

| Precursor | Plant | Incorporation (% × $10^{-2}$) |
|---|---|---|
| [2-$^{14}$C]Mevalonic acid [a] | *A. fruticosa* | 0.03 |
| [2-$^{14}$C]Mevalonic acid [b] | *D. elliptica* | 0.7 |
| [2-$^{3}$H]Mevalonic acid [c] | *D. elliptica* | $<0.004$ |
| [1-$^{3}$H]Isopentenyl alcohol [c] | *A. fruticosa* | 5.5 |
| [1-$^{3}$H]Dimethylallyl alcohol [c] | *A. fruticosa* | 0.19 |
| 3-Hydroxy-3-methyl-[3-$^{14}$C]-glutaric acid [c] | *A. fruticosa* | 0.12 |

[a] Ref. 1. [b] M. Hamada and M. Chubachi, *Agric. Biol. Chem.*, 1969, **33**, 793. [c] Present work.

using either *A. fruticosa* or *D. elliptica*, are poor, a situation by no means unique in biosynthetic investigations of meroterpenoids of higher plant origin.[16] Table 2 summarises data for incorporations of mevalonic acid and other prenyl precursors into amorphigenin (*A. fruticosa*) and rotenone (*D. elliptica*). [1-$^{3}$H]Isopentenyl alcohol gave the best incorporation into amorphigenin, but even here the figures were comparatively low (5.5 × $10^{-2}$ %). Since much better results were obtained with post-chalcone precursors in general, we have based this investigation on late-stage precursors using labelled rotenoids described in the previous paper,[17] particularly (*E*)-[4′-$^{14}$C]rot-2′-enonic acid and [7′-$^{14}$C]rotenone.

(*E*)-[4′-$^{3}$H]Rot-2′-enonic acid was administered to a large batch (1 200) of germinating *A. fruticosa* seeds with the aim of avoiding the need to add carrier rotenoids in the isolation procedures. Pure, labelled amorphigenin (5e) was isolated directly (118 mg) and showed a good incorporation figure (0.77%) (Table 3): this bears out our earlier observations in which a ring-*B*-labelled precursor was employed.[1] We were also able to isolate pure, labelled dalpanol (5c) (12.2 mg) from germinating *A. fruticosa* seeds for the first time. Although incorporation into dalpanol was lower than into amorphigenin (a

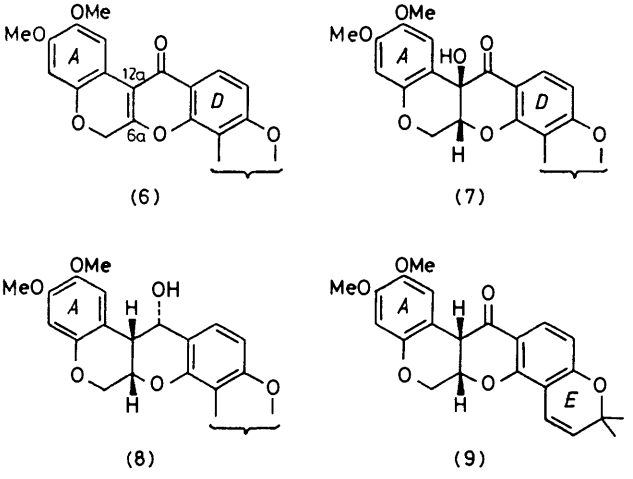
TABLE 3

Administration of (*E*)-[4′-$^3$H]rot-2′-enonic acid to *A. fruticosa* [a]

| | Rotenoid isolated | | |
|---|---|---|---|
| | Amorphigenin (5e) | Dalpanol (5c) | 12αβ-Hydroxyamorphigenin (7e) |
| Wt. isolated (mg) | 117.6 | 12.2 | 16.3 |
| Specific activity (disint. $min^{-1}$ $mmol^{-1}$) | $1.12 \times 10^6$ | $6.77 \times 10^6$ | $6.48 \times 10^6$ |
| Dilution | 866 | 143 | 150 |
| Incorporation (%) | 0.77 [b] | 0.54 [b] | 0.83 [b] |

[a] Precursor (14.6 mg, $3.55 \times 10^7$ disint. $min^{-1}$) applied to 1 200 seedlings for 46 h; uptake 83.3%. [b] Corrected for uptake.

consequence of the lesser quantity present) the specific activity was higher (*ca.* six-fold) and the dilution lower than for amorphigenin. These data are consistent with the position of dalpanol as a forerunner of amorphigenin.

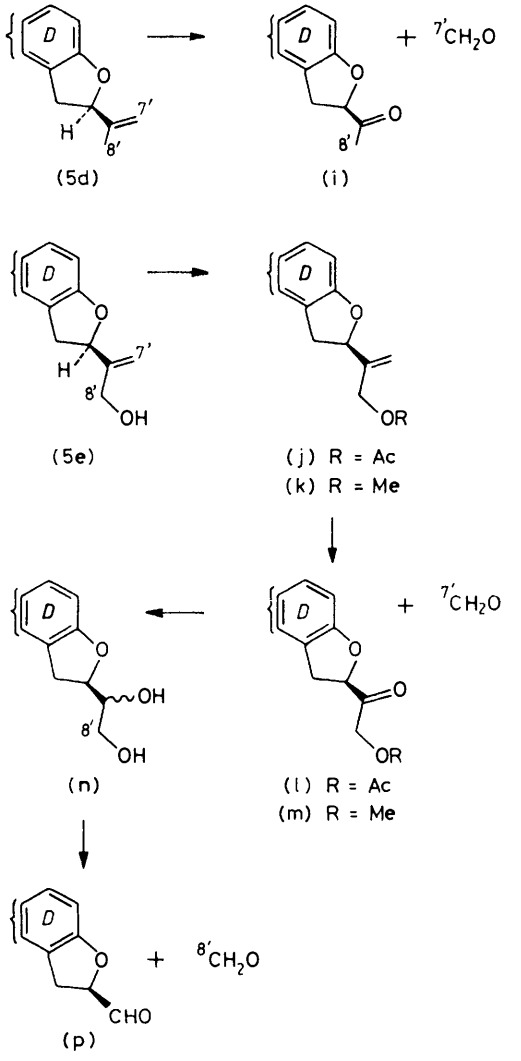



(6) (7) (8) (9)

A third compound (16.3 mg) obtained in this experiment proved, on spectroscopic examination, to be labelled 12aβ-hydroxyamorphigenin (7e) with specific activity close to that of dalpanol. This observation sheds light on the status of 12a-hydroxy-rotenoids in the plant. As mentioned earlier their formation during extraction of rotenoids under certain conditions always adds an element of uncertainty about their true occurrence in Nature. In the present case, the high specific activity of pure chiral 12aβ-hydroxyamorphigenin precludes its formation from amorphigenin, at least as the major source. It is clearly a genuine natural product, apparently originating by 12a-hydroxylation of dalpanol or an earlier metabolite, *i.e.* in a path such as (5c) ⟶ (7c) ⟶ (7d) ⟶ (7e). This raises the question of whether the same, or different, enzymes mediate reactions (5c) ⟶ (5e) and (7c) ⟶ (7e).

Prompted by the better incorporation of isopentenyl alcohol as compared with dimethylallyl alcohol (Table 2), and also reports on the biosynthesis of dihydrotremetone where an isopentenylated intermediate was thought to be implicated,[18] we have administered [4′-$^{14}$C]rot-3′-enonic acid (5h) [17b] to the *A. fruticosa system*. It was not metabolised to amorphigenin and we have no evidence that it mediates between (4) and (5a).

In approaching the stereochemical problems contained within the sequence (5a) ⟶ (5e), it seemed important to isolate the possible ambiguities inherent in the allylic hydroxylation step (5d) ⟶ (5e). The overall pathway was therefore monitored at the rotenone (5d) stage, using dilution methods for isolation. A specimen of [$^{14}$C]rot-2′-enonic acid having $^{14}$C distributed between C-4′ (88%) and C-5′ (12%) [17b] was administered to 1 000 *A. fruticosa* seedlings. After 48 h, radiolabelled rotenone was isolated and crystallised to constant activity. A specimen was degraded by oxidative cleavage [3] (Scheme 3) to provide the nor-ketone (5i) and formaldehyde. Details are given in Table 4. With 92% of the label accounted for, the label distribution in the rotenone, after correcting for $^{14}$C located at C-5′ in the precursor, is 91.3% in C-7′




SCHEME 3 Degradation of rotenone and amorphigenin for extraction of isotopic labels

TABLE 4

Administration of (*E*)-[4′-$^{14}$C]rot-2′-enonic acid to *A. fruticosa* [a]

| | |
|---|---|
| Specific activity of precursor | $1.12 \times 10^9$ disint. min$^{-1}$ mmol$^{-1}$ |
| Total activity of precursor | $1.01 \times 10^7$ disint. min$^{-1}$ |
| Uptake | 68.7% |
| Specific activity of rotenone [b] | $7.61 \times 10^4$ disint. min$^{-1}$ mmol$^{-1}$ |
| Incorporation (%) | 1.1% |

[a] Precursor (3.57 mg) applied to 1 000 seedlings for 48 h. [b] After addition of carrier (22.3 mg), purification, and dilution by a factor of 17.3.

(methylene) and 8.7% in C-8′ (methyl), a 10.6 : 1 ratio. It is apparent that there is high stereoselectivity in the overall conversion (5a) ⟶ (5d). Taking into account the use of a precursor having distributed labelling, and the need to isolate labelled rotenone by dilution methods,

TABLE 5

Activity of degradation products from rotenone (from Table 4)

| Compound | Specific activity (disint. min$^{-1}$ mmol$^{-1}$) | Relative activity |
|---|---|---|
| Rotenone (5d) | $7.61 \times 10^4$ | 1.00 |
| Formaldehyde dimedone | $5.60 \times 10^4$ | 0.7 |
| Diketone (5i) | $1.37 \times 10^4$ | 0.18 |

a moderately high experimental error is to be expected and it appears probable that the overall process is actually stereospecific. If so, it can be factorised into three stereospecific steps.

The first of these (5a) ⟶ (5b) might involve either α- or β-epoxidation of the 2′,3′-double bond, leading either to the (2′*S*,3′*S*)-epoxide (5b) or its 2′,3′-epimer. Assuming that the reaction (5b) ⟶ (5c) involves normal rear-side attack on C-2′ of the epoxide, then the stereochemistry shown (5b) must prevail in order to generate the well established 5′*R*-configuration of dalpanol and rotenone. It follows that the [7′-$^{14}$C]dalpanol intermediate would be a (5′*R*,6′*S*)-compound (5c). Dehydration of the hydroxyisopropyl centre must also be stereospecific, involving the 7′-labelled methyl of the 6′*S*-centre, which becomes the 7′-methylene of rotenone (5d). Further work is in hand to develop this approach. In particular more information is required on the postulated epoxide, which has not been isolated. A more complex pathway involving epoxide opening by an external nucleophile, followed by cyclisation with displacement, could require modification of the assignments. However, a suitable methodology for a more detailed examination of the problem is now provided by this work.

(iii) *Examination of the Regiospecificity of Hydroxylation of Rotenone to form Amorphigenin.*—The transformation of rotenone (5d) into amorphigenin (5e) can be viewed as a direct allylic oxidation, but the presence of a diol, amorphigenol (5f), in *Amorpha fruticosa* raises the question of an alternative pathway (5d) ⟶ (5f) ⟶ (5e). To evaluate the latter possibility, [7′-$^{14}$C]rotenone was oxidised to the pair of 6′-epimers (5f) using *N*-methylmorpholine *N*-oxide and a catalytic quantity of osmium tetraoxide.[19] N.m.r. indicates an approximate 3 : 1 mixture of epimers, though which predominates is not known. The [7′-$^{14}$C]-(6′*RS*)-amorphigenols were administered to *A. fruticosa* seedlings with the results summarised in Table 6. As expected, rotenone was an excellent precursor for amorphigenin, but although taken up

TABLE 6

Administration of [$^{14}$C]rotenoid precursors to *A. fruticosa*; formation of amorphigenin (5e)

| Precursor | Total activity of precursor (disint. min$^{-1}$) | Specific activity of amorphigenin (5e) (disint. min$^{-1}$ mmol$^{-1}$) | Incorporation (%) |
|---|---|---|---|
| [7′-$^{14}$C]Rotenone [a] | $1.19 \times 10^7$ | $7.86 \times 10^6$ | 1.89 |
| [7′-$^{14}$C]Rotenone [b] | $2.70 \times 10^7$ | $1.06 \times 10^6$ | 0.57 |
| [7′-$^{14}$C]-6′α- and 6′β-Amorphigenols (5f) [c] | $1.50 \times 10^6$ | 0 | 0 |

[a] Precursor (1.039 mg; $4.5 \times 10^9$ disint. min$^{-1}$ mmol$^{-1}$) applied to 450 seedlings for 48 h; uptake 97%. [b] Precursor (1.26 mg; $8.44 \times 10^9$ disint. min$^{-1}$ mmol$^{-1}$) applied to 1 250 seedlings for 48 h; uptake 96%. [c] Precursor (0.48 mg; $1.34 \times 10^9$ disint. min$^{-1}$ mmol$^{-1}$) applied to 200 seedlings for 48 h; uptake 69%.

by the seedlings, it is apparent that neither 5′-epimer of (5f) is metabolised to amorphigenin. A direct hydroxylation (5d) ⟶ (5e) remains the most likely route.

To track the fate of the rotenone 7′-$^{14}$C label, a method was required for the extraction of C-7′ and C-8′ in amorphigenin, and this is shown in Scheme 3. Preliminary experiments indicated that the label was being scrambled between C-7′ and C-8′ of (5e), and three different procedures were used because of the danger of generating a symmetrical allyl species during work up and degradation. The results are shown in Table 7 and relate to

TABLE 7

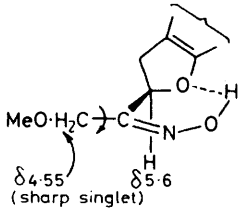
Degradation of $^{14}$C-labelled amorphigenin arising from Table 6 administrations of [7′-$^{14}$C]rotenone

| | Specific activity (disint. min$^{-1}$ mmol$^{-1}$) | Relative activity |
|---|---|---|
| Experiment A | | |
| Amorphigenin (5e) | $1.31 \times 10^6$ | 1.00 |
| Acetate (5j) | $1.31 \times 10^6$ | 1.00 |
| 7′-Formaldehyde dimedone | $6.23 \times 10^5$ | 0.48 |
| Ketol acetate (5l) | $6.31 \times 10^5$ | 0.48 |
| 8′-Formaldehyde dimedone | $7.06 \times 10^5$ | 0.54 |
| Experiment B | | |
| Amorphigenin (5e) | $1.31 \times 10^6$ | 1.00 |
| Methyl ether (5k) | $1.33 \times 10^6$ | 1.01 |
| 7′-Formaldehyde dimedone | $6.16 \times 10^5$ | 0.47 |
| Oxime of ketol (5m) | $6.23 \times 10^5$ | 0.48 |
| Experiment C | | |
| Methyl ether (5k) | $9.67 \times 10^5$ | 1.00 |
| 7′-Formaldehyde dimedone | $4.29 \times 10^5$ | 0.44 |
| Oxime of ketol (5m) | $4.62 \times 10^5$ | 0.48 |

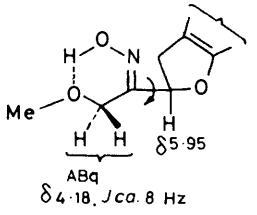
the amorphigenin produced from labelled rotenone in Table 6. In Experiment A, amorphigenin was extracted with aqueous 95% ethanol, purified by p.l.c. on silica, and

acetylated with acetic anhydride–pyridine at 20 °C to form (5j). Oxidative cleavage (osmium tetraoxide–periodate) provided the oxo-acetate (5l) together with C-7′ as formaldehyde, isolated as the dimedone derivative. Reduction of (5l) by lithium aluminium hydride gave the triol (8n), which consists of two epimers. For characterisation purposes these have been separated, but in radiochemical experiments the mixture was cleaved directly with periodate to yield the rotenoid aldehyde (8p), together with C-8′ as formaldehyde (dimedone derivative). Counting data (Table 7) indicate an equal distribution of label between C-7′ and C-8′.

The possibility that labelling on C-7′ and C-8′ was originally different, but became equivalent during acetylation of the allylic system, was considered. Carbocation formation or [3,3]sigmatropic shifts are capable of bringing this about; so too could an $S_N2'$ acetoxy-interchange process. A second experiment (Experiment B) in which the methyl ether (5k) was employed was therefore designed. Here the ether was formed in low polarity medium (MeI–$Ag_2O$–$CHCl_3$). Oxidative cleavage again gave C-7′ as formaldehyde and the oxo-ether (5m), which was purified as the crystalline oxime. [This formed as a mixture of *Z*- and *E*-oximes which were separated and characterised. Our suggested assignments of their geometry will be evident from the n.m.r. data appended to the diagrams for (*Z*)-(10) and (*E*)-(10) given in Scheme 4. Only the more easily




(*Z*)-(10), m.p. 148–150°C (higher $R_F$)

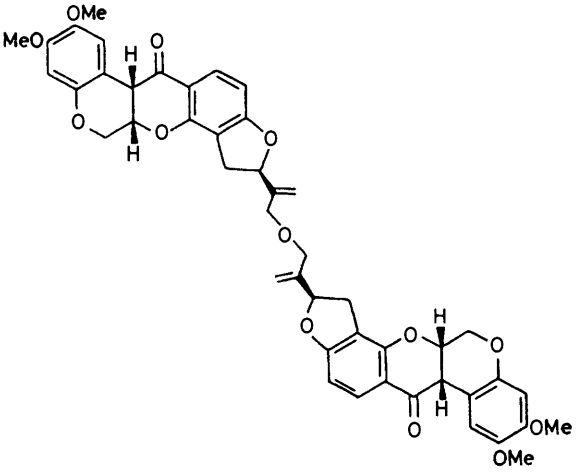



(*E*)-(10), m.p. 180–182°C (lower $R_F$)

SCHEME 4 Assignments of oxime stereochemistry

crystallising *E*-isomer was used for counting purposes.] Again, the result (Table 7) was an equal distribution of label between C-7′ and C-8′. Finally, in Experiment C, amorphigenin was isolated by dilution and recrystallisation, thus avoiding any possibility of scrambling of the allylic alcohol while adsorbed on silica during chromatographic operations. The methyl ether was formed and degraded as before. Once more the label was equally shared between C-7′ and C-8′.

Thus in these experiments, contrary to our initial expectations, the labelled C-7′ in rotenone becomes at some stage chemically equivalent to C-8′. Allylic rearrangement during extraction appears unlikely, particularly since there is no evidence that the solvent (95% ethanol–water) participates in a chemical reaction (we have not observed the formation of 8′-ethoxyrotenone). There remain various biochemical possibilities. The biological hydroxylation of rotenone may involve a symmetrical allyl species but in the absence of studies using isolated enzyme preparations it is premature to reach this conclusion. A regiospecific hydroxylation could be followed by an *in vivo* scrambling. Free and bound forms of amorphigenin are involved in active metabolism in *A. fruticosa* seedlings and there are opportunities for mechanisms which could involve equilibration




(11)

of the allylic positions. Further experimentation will be needed to evaluate the biochemical significance of these results on the hydroxylation process that leads from rotenone to amorphigenin.

Bulk supplies of amorphigenin for this work were obtained by hydrolysis of its glycoside amorphin, obtained from ungerminated *A. fruticosa* seeds. Dilute sulphuric acid, ion-exchange resin or β-glucosidase can be employed.[6] When 5.5M-hydrochloric acid was used, certain crystalline by-products were noted. One was 8′-chlororotenone, m.p. 162—164 °C, and another proved to be the anhydro-dimer (11), m.p. 215—218 °C, $M^+$, 802. Details are given in the Experimental section.

## EXPERIMENTAL

For general procedures see the accompanying paper.[17a]

*Growth of* Amorpha fruticosa *Seedlings and Administration of Precursors.*—Dry *A. fruticosa* seeds were dehusked mechanically between rotating glass-paper discs in a specially constructed apparatus. Husk debris was removed by sieving (0.75 mm mesh) and water flotation. The seeds were then sterilised by washing with ethanol, aqueous 8% copper

sulphate, and water. After soaking in water at 50 °C for 10 min, seeds were set aside to germinate. Seeds of high viability were germinated in clean covered boxes without special treatment, but for batches with low viability (<40%), all equipment was sterilised, and a sterile cabinet was employed: germinated seeds were rinsed in dilute aqueous copper sulphate and removed to separate containers. Fungal infections were thus prevented. Seedlings of comparable size were selected after *ca.* 7 days and grown on in sterile Petri dishes with illumination (quartz-halogen lamps). Water-soluble precursors were administered in 0.05M-phosphate buffer: water-insoluble compounds were dissolved in methyl cellosolve (*ca.* 0.1 $cm^3$), Tween 20 (*ca.* 40 $mm^3$) was added, and the solution was diluted with water or 0.05M-phosphate buffer (pH 7.0) with further Tween 20 if required. All precursors were administered in a single dose, and seedlings were grown on for 48 h. Unabsorbed precursor was removed by washing, and counted.

*Extractions of* A. fruticosa.—(a) *Amorphigenin from seedlings.* A typical procedure for 200 seedlings is as follows. Plant material was ground under ethanol–water (1 : 1; 10 $cm^3$) with the aid of broken glass. The mixture was refluxed for 10 min with ethanol (100 $cm^3$), then filtered, and the residue was twice extracted with ethanol. Combined extracts were evaporated, and the green residue was taken up in water (50—75 $cm^3$). The aqueous mixture was sequentially extracted with light petroleum (3 × 50 $cm^3$), ether (3 × 50 $cm^3$), and chloroform (3 × 50 $cm^3$). The combined ether and chloroform extracts were dried and evaporated. The residual gum, mainly amorphigenin, was purified by p.l.c. with (i) chloroform–propan-2-ol (20 : 1) ($R_F$ 0.52; double development, yielding crystalline product) and (ii) benzene–ethyl acetate–methanol–light petroleum (b.p. 60—80 °C) (6 : 4 : 1 : 3). Crystallisation from methanol was effected after the addition of carrier material, to yield amorphigenin methanol solvate, m.p. 196—197 °C. Amorphigenin content of the seedlings naturally varied but the average was *ca.* 75 × $10^{-6}$ g per seedling.

(b) *Dalpanol* (5c) *and* 12aβ-*hydroxyamorphigenin* (7e).—3 500 Seedlings were extracted as in (a). The ether–chloroform extracts on t.l.c. examination (chloroform–propan-2-ol, 20 : 1) showed yellow fluorescent (366 nm radiation) spots at $R_F$ 0.75 and 0.46, in addition to the amorphigenin spot. P.l.c. gave, from the higher $R_F$ band, dalpanol (56.5 mg; 16 × $10^{-6}$ g per seedling), m.p. and mixed m.p. with authentic dalpanol 192—193 °C, and with mass, u.v., i.r., and $^1H$ n.m.r. spectra concordant with this structure. The lower $R_F$ band gave 12aβ-hydroxyamorphigenin as an amorphous solid (52.9 mg, 15 × $10^{-6}$ g per seedling), ' m.p. ' 92—95 °C, $[\alpha]_D^{24}$ −175° (*c* 1.8, $CHCl_3$), {lit.,[20] m.p. 94—96 °C (glass), $[\alpha]_D^{20}$ −181°}; *m/z* 426 ($M^+$); $\lambda_{max.}$ (EtOH) 237 (4.11), 245infl (4.15), and 292 nm (4.41); $\nu_{max.}$ (KBr) 3 450, 1 675, 1 612, 1 515, 822, and 748 $cm^{-1}$; δ ($CDCl_3$; 60 MHz) 7.91 (1 H, d, *J* 9 Hz, 11-H), 6.56 (1 H, d, *J* 9 Hz, 10-H), 6.63 (1 H, s, 1-H), 6.53 (1 H, s, 4-H), 5.42 (1 H, t, 5′-H), 5.26 (2 H, s, 7′-$H_2$), 4.57 (3 H, m 6a-H, 6-$H_2$), 4.25 (2 H, s, 8′-$H_2$), 3.82 and 3.72 (each 3 H, s, OMe), and 3.20 (2 H, m, 4′-$H_2$) [two s at 3.82 and 2.34 (2 × OH) disappeared after $D_2O$ treatment]. These data agree with literature assignments.

(c) *Amorphin from* A. fruticosa *seeds.* Ground seeds (1 kg) were extracted (Soxhlet) with dry light petroleum (b.p. 40—60 °C) for 36 h. The dried marc was re-extracted with methylene chloride for 36 h, re-dried, and finally extracted with dry chloroform–methanol (9 : 1). The last extract was evaporated and the residue triturated under hot benzene. The resulting brown solid was recrystallised twice from aqueous methanol to yield amorphin (18 g), m.p. 154 °C, $[\alpha]_D$ −118° (*c* 1.0, MeOH) {lit.,[6] m.p. 154—155 °C, $[\alpha]_D$ −123.6° (MeOH)}.

(d) 6a,12a-*Didehydrorotenoids from extraction mother liquors.* Methanolic extracts of *A. fruticosa* seeds, from which much amorphin had been removed, slowly deposited solid material which was collected, washed with hot chloroform, and hydrolysed with dilute (12%) hydrochloric acid at reflux for 1 h. The solid precipitate was collected, dried, and separated by p.l.c. (chloroform–propan-2-ol, 10 : 1). A band of $R_F$ 0.71 yielded 6a,12a-didehydrodalpanol (6c) (15 mg), m.p. 238—240 °C (lit.,[5] 238—240 °C); *m/z* 410 ($M^+$), with spectroscopic data in agreement with literature values. A band at $R_F$ 0.64 gave 6a,12a-didehydroamorphigenin (6e) (45 mg), m.p. 227—229 °C, not depressed by admixture with authentic material, m.p. 227—229 °C.[6] A third band gave yellow needles (10.7 mg) of 6a,12a-*didehydro*-6′,7′-*dihydro*-6′,7′-*dihydroxyrotenone* (6f) (didehydroamorphigenol), m.p. 240 °C (Found: C, 64.2; H, 4.95%; $M^+$, 426. $C_{23}H_{22}O_8$ requires C, 64.8; H, 5.15%; *M*, 426); $\lambda_{max.}$ 240 (4.37), 246 (4.37), 280 (4.31), and 312 nm (4.21); $\nu_{max.}$ (KBr) 3509, 3 420, 1 635, 1 612, 1 512, 871, and 783 $cm^{-1}$, δ (100 MHz; $CDCl_3$–$CD_3SOCD_3$) 8.44 (1 H, s, 1-H), 8.05 (1 H, d, *J* 9 Hz, 11-H), 6.90 (1 H, d, *J* 9 Hz, 10-H), 6.57 (1 H, s, 4-H), 5.06 (1 H, 5′-H), 5.02 (2 H, s, 6-$H_2$), 3.94 and 3.88 (each 3 H, s, OMe), 3.62 (2 H, 4′-$H_2$), and 2.64 (3 H, s, 8′-$H_3$). Finally, a band at $R_F$ 0.10 yielded 6a,12a-didehydroamorphin (1.8 mg), m.p. and mixed m.p. with an authentic sample made by dehydrogenating amorphin, 180—182 °C.

(e) 6*a*,12*a*-*Didehydrorotenoid glycosides.* The extracts used in (d) above were fractionated by p.l.c. (chloroform–methanol, 4 : 1). Three major components, at $R_F$ 0.75, 0.45, and 0.27, were isolated and crystallised from chloroform–methanol–water. Each sample was hydrolysed (aq. HCl): the sugar content of neutralised, concentrated extract was analysed by paper chromatography (butan-1-ol–acetic acid–water, 4 : 1 : 5), using silver nitrate for location, and standard carbohydrate references. The material of $R_F$ 0.75 yielded 6a,12a-didehydrodalpanol, m.p. 238—240 °C, glucose, and arabinose on hydrolysis. Field desorption mass spectrometry showed ions at *m/z* 704 (11%) and 574 (100%), suggesting that the product was mainly 6a,12a-didehydrodalpanol glucoside with a minor proportion of the corresponding vicianoside. In support, the $^1H$ n.m.r. spectrum showed signals appropriate to 6a,12a-didehydrodalpanol, with superimposed carbohydrate proton signals corresponding to *ca.* 75% glucoside, 25% vicianoside. The sample at $R_F$ 0.45, m.p. 145—153 °C, gave on hydrolysis 6a,12a-didehydroamorphigenol, m.p. 240 °C, glucose, and small traces of arabinose and an unidentified sugar: thus this product appears to be mainly 6a,12a-didehydroamorphigenol glucoside, *m/z* 588 ($M^+$), showing $^1H$ n.m.r. signals of the rotenoid and the carbohydrate residue. Finally the band at $R_F$ 0.27 gave 6a,12a-didehydroamorphin, m.p. 180—182 °C, glucose, and arabinose, the field desorption mass spectrum showing ions at 702 (100%) and 572 (45%), and the $^1H$ n.m.r. integrations suggesting that a proportion of 6a,12a-didehydroamorphigen glucoside was also present. The n.m.r. showed the relatively undisturbed rotenoid resonances with superimposed carbohydrate signals.

*Hydrolysis of Amorphin by Hydrochloric Acid.*—Amorphin (6.1 g) was suspended in 5.5M-hydrochloric acid (500 $cm^3$)

and the mixture was refluxed for 2 h. The solid was filtered off and washed with chloroform, and the chloroform-insoluble material refluxed for a further 4 h in acid. The solid was collected and washed with chloroform. The chloroform-soluble material was recovered by evaporation, and passed through a short alumina column in chloroform–propan-2-ol (20 : 1). The product (1.4 g) was separated by p.l.c. to yield amorphigenin (0.48 g), m.p. 188—189 °C, mixed m.p. 190 °C with an authentic specimen, and with the same $^1$H n.m.r. spectrum as authentic rotenoid. In addition, *8'-chlororotenone* (0.2 g), m.p. 162—164 °C, $[\alpha]_D^{27}$ −97° (*c* 0.08, $CHCl_3$), was isolated (Found: C, 64.15; H, 4.7; Cl, 7.9%; $M^+$, 428.101. $C_{23}H_{21}O_6Cl$ requires C, 64.5; H, 4.9; Cl, 8.3%; *M*, 428.101); $\nu_{max.}$ (KBr) 1 670 and 1 610 cm$^{-1}$; $\lambda_{max.}$ ($CHCl_3$) 291 (4.31) and 313 nm (4.02); δ ($CDCl_3$) 7.86 (1 H, d, *J* 8 Hz, 11-H), 6.79 (1 H, s, 1-H), 6.54 (1 H, d, *J* 8 Hz, 10-H), 6.47 (1 H, s, 4-H), 5.45 (1 H, 5'-H), 5.41 (2 H, d, 7'-$H_2$), 4.86 (1 H, m, 6a-H), 4.40 (2 H, m, 6-$H_2$), 4.20 (2 H, s, 8'-$H_2$), 3.87 (1 H, d, *J* 4 Hz, 12a-H), 3.83 and 3.79 (each 3 H, s, OMe), and 3.30 (2 H, m, 4'-$H_2$). In a higher $R_F$ fraction was found the *bis-rotenoid ether* (11) (0.2 g), m.p. 215—218 °C, $[\alpha]_D^{27}$ −142° (*c* 0.08, $CHCl_3$) (Found: $M^+$, 802.265. $C_{46}H_{42}O_{13}$ requires *M*, 802.263); $\nu_{max.}$ (KBr) 1 675 and 1 610 cm$^{-1}$; δ ($CDCl_3$; 220 MHz), 7.84 (1 H, d, *J* 10 Hz, 11-H), 6.77 (1 H, s, 1-H), 6.51 (1 H, d, *J* 10 Hz, 10-H), 6.46 (1 H, s, 4-H), 5.31 (1 H, 5'-H), 5.30 and 5.25 (each 1 H, s, 7'-$H_2$), 4.90 (1 H, m, 6a-H), 4.40 (2 H, m, *J* 3 and 12 Hz, 6-$H_2$), 4.10 (2 H, ABq, *J* 12.5 Hz, 8'-$H_2$), 3.81 and 3.78 (both 3 H, s, 2 × OMe), and 3.20 (2 H, 4'-$H_2$).

*Acetylation of Amorphigen.*—Amorphigen (118 mg) in dry acetic anhydride (20 cm$^3$) and dry pyridine (0.1 cm$^3$) was set aside overnight. The solution was stirred with ice and water for 90 min, and the mixture extracted with chloroform (5 × 10 cm$^3$). Combined extracts were washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated to yield amorphigenin acetate (101 mg), m.p. 159—161 °C (lit.,[6] 166—167 °C: possibly a second crystalline form); the sample was spectroscopically indistinguishable from authentic material.

*Oxidative Cleavage of Amorphigenin Acetate.*—Amorphigenin acetate (97 mg) in tetrahydrofuran * (freshly distilled from lithium aluminium hydride; 3.5 cm$^3$) and water (1.5 cm$^3$) was stirred with osmium tetraoxide (*ca.* 5 mg) for 10 min. Powdered sodium periodate (200 mg) was added, and the mixture stirred for 3.5 h, then diluted with water (20 cm$^3$), and distilled. The distillate was collected in aqueous dimedone (15 cm$^3$; 3 g dm$^{-3}$); the solution was kept at 20 °C for 1 h, and overnight at 0 °C. The dark precipitate was collected by filtration and dried, and osmium dioxide was removed by dissolving the solid in chloroform and filtering. The resulting clear chloroform solution was evaporated to yield the dimedone derivative of formaldehyde (45 mg, 70%), m.p. 190—191 °C, m.p. and mixed m.p. with an authentic sample, 189—190 °C. In radiochemical work the sample was recrystallised from ethanol to constant activity.

The residue from distillation was extracted with chloroform. The extracts after washing, drying, and evaporation gave a semicrystalline mass which after p.l.c. [light petroleum (b.p. 60—80 °C)–chloroform–propan-2-ol, 5 : 9 : 1] yielded the *oxo-acetate* (5l) (63 mg), m.p. 195—197 °C (Found: C, 62.95; H, 4.85. $C_{24}H_{22}O_9$ requires C, 63.45; H, 4.85%); $\lambda_{max.}$ 233 (4.18), 243infl (3.97), 289 (4.20), and 313infl nm (3.92); $\nu_{max.}$ (KBr) 2 965, 1 748, 1 715, 1 677, 1 638, and 1 515 cm$^{-1}$; δ ($CDCl_3$) 7.79 (1 H, d, *J* 9 Hz, 11-H), 6.67 (1 H, s, 1-H), 6.50 (1 H, d, *J* 9 Hz, 10-H), 6.38 (1 H, s, 4-H), 5.22 (1 H, 5'-H), 4.90 (1 H, m, 6a-H), 4.90 (2 H, s, 8'-$H_2$), 4.57 (1 H, dd, *J* 3 and 12 Hz, 6-$H_a$), 4.09 (1 H, d, *J* 12 Hz, 6-$H_b$), 3.77 (1 H, 12a-H), 3.77 (3 H, s, OMe), 3.72 (3 H, s, OMe), 3.34 (2 H, 4'-$H_2$), and 2.13 (3 H, s, COMe). For further crystallisation, for radiochemical purposes, we used chloroform–ether.

*Lithium Aluminium Hydride Reduction of the Oxo-acetate* (5 l).—The oxo-acetate (25 mg) was dissolved in dry tetrahydrofuran (15 cm$^3$) and lithium aluminium hydride (15 mg) was added. The mixture was stirred at ambient temperature for 3 h then aqueous ammonium chloride was added. The product was filtered and evaporated. The residue was purified by p.l.c., first by triple development in light petroleum (b.p. 40—60 °C)–ether–ethyl acetate (3 : 3 : 1), and then by double development in light petroleum (b.p. 40—60 °C)–ether–ethyl acetate–methanol (6 : 6 : 2 : 1) The two epimers of the *triol* (8n) were thus separated. The higher $R_F$ form was obtained as a glass (7.1 mg, 31%) (Found: $M^+$, 416.146. $C_{22}H_{24}O_8$ requires *M*, 416.147), $\lambda_{max.}$ 288 nm (3.80); $\nu_{max.}$ ($CHCl_3$) 3 500, 2 986, 1 622, and 1 098 cm$^{-1}$; δ ($CDCl_3$–$CD_3SOCD_3$) 7.03 (1 H, d, *J* 9 Hz, 11-H), 6.69 (1 H, s, 1-H), 6.43 (1 H, s, 4-H), 6.41 (1 H, d, *J* 9 Hz, 10-H), 5.24 (1 H. m, 5'-H), 5—3.5 (8 H, complex, 6-$H_2$, 6a-H, 12a-H, 12-H, 6'-H, 7'-$H_2$), 3.80 and 3.77 (each 3 H, s, 2 × OMe), and 3.01 (2 H, 4'-$H_2$). The lower $R_F$ form was also a glass (7.7 mg, 33%) (Found: $M^+$, 416.145. $C_{22}H_{24}O_8$ requires *M*, 416.147); $\lambda_{max.}$ 288 nm (3.80); $\nu_{max.}$ ($CHCl_3$) 3 500, 1 620, and 984 cm$^{-1}$; δ ($CDCl_3$–$CD_3SOCD_3$) 7.03 (1 H, d, *J* 9 Hz, 11-H), 6.70 (1 H, s, 1-H), 6.43 (1 H, s, 4-H), 6.41 (1 H, d, *J* 9 Hz, 10-H), 5.26 (1 H, m, 5'-H), 5—3.5 (8 H, complex, 6-$H_2$, 6a-H, 12a-H, 12-H, 6'-H, 7'-$H_2$), 3.80 and 3.77 (each 3 H, s, 2 × OMe), and 3.00 (2 H, 4'-$H_2$). In radiochemical work the epimers were not separated but isolated from p.l.c. (chloroform–propan-2-ol, 4 : 1) as one band (60%) and immediately treated with sodium periodate as described below. The triols were unstable in solution.

*Oxidative Cleavage of the Triol* (8n).—The triol (8n) (19 mg) in freshly distilled tetrahydrofuran (2.5 cm$^3$) and water (1 cm$^3$) was stirred with powdered sodium periodate (40 mg) at ambient temperature for 3 h. Water (20 cm$^3$) was added and the mixture distilled into dimedone reagent (8 cm$^3$; 3 g dm$^{-3}$). The aqueous product was set aside for 1 h at 20 °C and overnight at 0 °C, then filtered to yield the derivative of formaldehyde as above (5.7 mg), m.p 190—191 °C.

*8'-Methoxyrotenone.*—Amorphigenin (83 mg) in chloroform (10 cm$^3$; distilled and then shaken over basic alumina for 12 h to remove acid), methyl iodide (200 mm$^3$), and finely-ground silver oxide (1.8 g) were stirred together at 55—60 °C for 2.5 h: more methyl iodide (200 mm$^3$) was added, and the reaction continued for 2 h more. The mixture was filtered. Evaporation of the filtrate afforded O-*methylamorphigenin* (5k) (62.3 mg, 73%), m.p. 182—185 °C (from chloroform–methanol), $[\alpha]_D^{27}$ −114° (*c* 0.8, $CHCl_3$) (Found: C, 67.65; H, 5.6%; $M^+$, 424.141. $C_{24}H_{24}O_7$ requires C, 67.9; H, 5.65%; *M*, 424.152); $\lambda_{max.}$ ($CHCl_3$) 294 nm (4.29); $\nu_{max.}$ (KBr) 1 670 and 1 607 cm$^{-1}$; δ ($CDCl_3$) 7.84 (1 H, d, *J* 8 Hz, 11-H), 6.80 (1 H, s, 1-H), 6.50 (1 H, d, *J* 8 Hz, 10-H), 6.48

* Difficulties were encountered when using dioxan, a common solvent for the reaction. Although this solvent was purified by refluxing overnight with aqueous hydrochloric acid in nitrogen, followed by refluxing over, and distillation from, sodium, control reactions showed that unlabelled formaldehyde was being generating, thus leading to counting errors.

(1 H, s, 4-H), 5.30 (3 H, m, 5′-H and 7′-$H_2$), 4.90 (1 H, m, 6a-H), 4.35 (2 H, m, $J$ 4 and 11 Hz, 6-$H_2$), 4.05 (2 H, s, 8′-$H_2$), 3.85 (1 H, 12a-H), 3.90 and 3.85 (each 3 H, s, 2 × OMe), 3.44 (3 H, s, 8′-OMe), and 3.18 (2 H, m, 4′-$H_2$).

*Oxidative Cleavage of 8′-Methoxyrotenone.*—The ether (66 mg) in tetrahydrofuran (4 $cm^3$) and water (1.5 $cm^3$) was stirred with osmium tetraoxide (*ca.* 5 mg) for 10 min. Following the procedure in the similar experiment above gave the dimedone derivative of formaldehyde (16.3 mg, 35%), and the *methoxy-ketone* (5m) (35 mg, 53%) as an amorphous solid (Found: $M^+$, 426.131. $C_{23}H_{22}O_6$ requires $M$, 426.128); $\nu_{max.}$ (KBr) 1 730, 1 670, and 1 610 $cm^{-1}$; δ ($CDCl_3$) 7.98 (1 H, d, $J$ 8 Hz, 11-H), 6.85 (1 H, s, 1-H), 6.65 (1 H, d, $J$ 8 Hz, 10-H), 6.55 (1 H, s, 4-H), 5.39 (1 H, 5′-H), 5.00 (1 H, 6a-H), 4.41 (2 H, m, $J_{AB}$ 10, $J_{6,6a}$ 4 Hz, 6-$H_2$), 4.40 (2 H, s, 7′-$H_2$), 3.90 (1 H, 12a-H), 3.88 and 3.85 (each 3 H, s, 2 × OMe), 3.53 (3 H, s, 7′-OMe), and 3.41 (2 H, 4′-$H_2$). The ketone (5m) (36 mg) in tetrahydrofuran (2 $cm^3$) was treated with hydroxylamine hydrochloride (7.0 mg) and sodium acetate (8.5 mg) in water (1 $cm^3$) and ethanol (2 $cm^3$). The mixture was refluxed for 30 min, and then evaporated. The residue was partitioned between water and chloroform. Chloroform extracts were dried and evaporated, and the product was separated into the geometric isomers of the *oxime* of (5m) by p.l.c. (double development in chloroform–propan-2-ol, 20 : 1). The *higher* $R_F$ *isomer*, (*Z*)-(10) (6.8 mg, 18%) was crystallised from methanol with difficulty, m.p. 148—150 °C (microcrystalline powder) (Found: $M^+$, 441.141. $C_{23}H_{23}NO_8$ requires $M$, 441.142); $\lambda_{max.}$ ($CHCl_3$) 318 nm (3.59); $\nu_{max.}$ (KBr) 3 450, 1 675, and 1 615 $cm^{-1}$; δ ($CDCl_3$) 7.90 (1 H, d, $J$ 8 Hz, 11-H), 6.83 (1 H, s, 1-H), 6.55 (1 H, d, $J$ 8 Hz, 10-H), 6.50 (1 H, s, 4-H), 5.60 (1 H, 5′-H), 4.95 (1 H, m, 6a-H), 4.55 (2 H, $J_{AB}$ 12, $J_{6.6a}$ 4 Hz, 6-$H_2$), 4.55 (2 H, s, 7′-$H_2$), 3.90 (1 H, 12a-H), 3.85 and 3.82 (each 3 H, s, 2 × OMe), 3.45 (3 H, s, 7′-OMe), and 3.45 (2 H, 4′-$H_2$). The *lower* $R_F$ *isomer*, (*E*)-(10) (12.5 mg, 34%), had m.p. 180—182 °C (from methanol) (Found: $M^+$, 441.143); $\lambda_{max.}$ ($CHCl_3$) 292 (4.26) and 315 nm (3.91); $\nu_{max.}$ (KBr) 3 450, 3 350, 1 670, and 1 610 $cm^{-1}$; δ ($CDCl_3$) 7.90 (1 H, d, $J$ 8 Hz, 11-H), 6.80 (1 H, s, 1-H), 6.55 (1 H, d, $J$ 8 Hz, 10-H), 6.50 (1 H, s, 4-H), 5.95 (1 H, 5′-H), 4.95 (1 H, m, 6a-H), 4.40 (2 H, 6-$H_2$), 4.18 (2 H, ABq, 7′-$H_2$), 3.85 and 3.80 (each 3 H, s, 2 × OMe), 3.50 (2 H, 4′-H), and 3.30 (3 H, s, 7′-OMe). In radiochemical work only the (readily crystallised) lower $R_F$ isomer was crystallised to constant activity.

6′,7′-*Dihydro*-6′7′-*dihydroxyrotenone* (5f).—Rotenone (17.4 mg) in tetrahydrofuran (3 $cm^3$) and water (1 $cm^3$) was treated with osmium tetraoxide (2 mg). After 10 min, *N*-methylmorpholine *N*-oxide (30 mg) was added and the mixture stirred for 7.5 h. The solution was then stirred with acidified sodium disulphite solution for 30 min, filtered, and evaporated. The residue was purified by p.l.c. (chloroform–methanol, 20 : 1) and crystallised from aqueous ethanol to yield the epimers of the diol (5f) (9.2 mg), m.p. 187—189 °C (lit.,[3] 199—201 °C, 197—198 °C for diols of unspecified epimeric content). The $^1H$ n.m.r. spectrum ($CDCl_3$) showed resonances for 8′-$H_3$ at δ 1.19 and 1.22 (3 : 1). A [7′-$^{14}$C]-specimen was made by this method.

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