Formation and Dehydration of a Prochiral 2-Hydroxyisopropyl Centre during Biosynthesis: the Rot-2′-enonic Acid–Rotenone Transformation in *Amorpha fruticosa*

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Summary Rot-2′-enonic acid (**2**), dalpanol (**4**), and rotenone (**5**) are formed during the conversion of 9-demethylmunduserone (**1**) into amorphigenin (**6**) by *Amorpha fruticosa* seedlings: the 4′-*E*-methyl of (**2**) becomes predominantly the 7′-methylene of (**5**), leading to stereochemical proposals for a formation scheme.

(6a*S*,12a*S*)-9-DEMETHYLMUNDUSERONE (**1**) is a key precursor for amorphigenin (**6**) biosynthesis by *Amorpha fruticosa* seedlings.[1] Circumstantial and other evidence suggests that the transformation may then proceed *via* prenylation to rot-2′-enonic acid (**2**) followed by oxidative cyclisation to dalpanol (**4**), probably *via* an intervening epoxide (**3**). Dehydration to rotenone (**5**) and hydroxylation to amorphigenin (**6**) is then envisaged as in the Scheme. We now demonstrate the presence of intermediates (**2**), (**4**), and (**5**) of the Scheme and examine the fate of a 4′-*E*-^{14}C-label in rot-2′-enonic acid during its conversion into rotenone.

A single dose of [2-^{14}C]phenylalanine was applied to batches of *A. fruticosa* seedlings and metabolites (**2**), (**4**), (**5**), and (**6**) were isolated after various intervals by isotope dilution, and crystallised to constant activity. Results, selected from the full study, showing the presence and labelling of the postulated intermediates are given in Table 1. Of these, rot-2′-enonic acid (**2**) was metabolised fairly rapidly and could not be found in incubations of 24 h or more, detection requiring shorter feeding times. [4′-^{14}C]-Rot-3′-enonic acid (**7**), however, was not metabolised to amorphigenin by the seedling system showing that it does not mediate between (**1**) and (**2**) despite the better incorporation found for isopentenyl alcohol rather than dimethylallyl alcohol.[2] Table 2 gives the results of an experiment

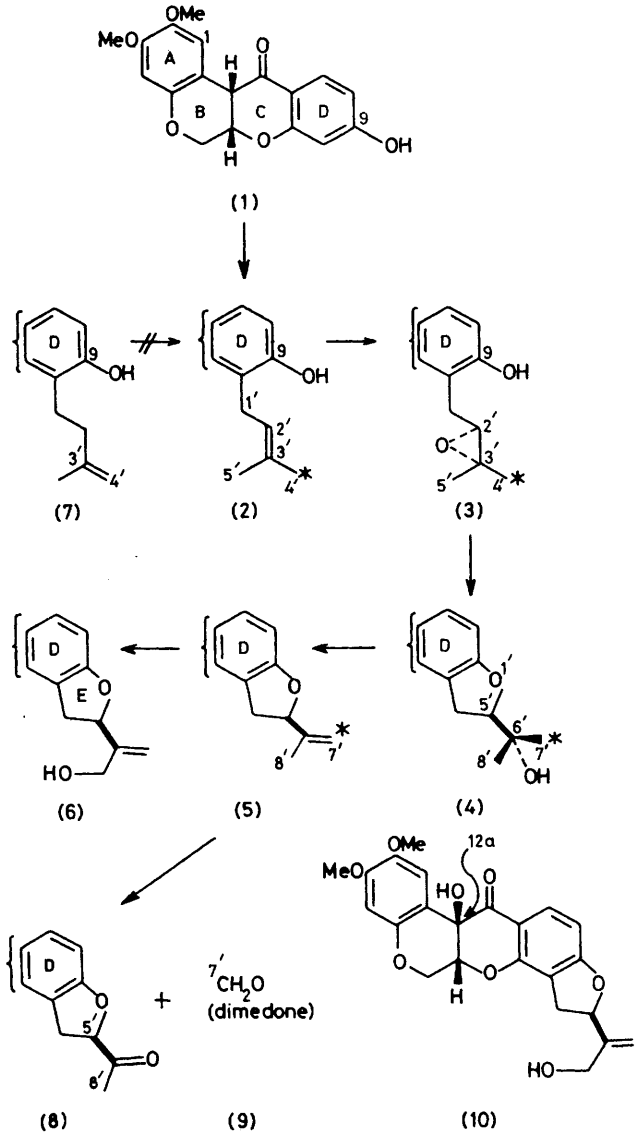
TABLE 1. Incorporation of [2-^{14}C]phenylalanine into rotenoids by *A. fruticosa* seedlings

Rotenoid isolated	Administration time/h	Incorporation /%
(**6**)	48	0·48
(**5**)	48	0·09
(**4**)	48	0·012
(**2**)	48	$<4 \times 10^{-4}$
(**2**)	11	0·03
(**2**)	6	0·02

TABLE 2. Incorporation of (*E*)-[4′-^{3}H]rot-2′-enonic acid[a] by *A. fruticosa* seedlings

	Rotenoids isolated		
	(**6**)	(**4**)	(**10**)
Wt. isolated/mg	117·6	12·2	16·3
Sp activity/d.p.m. mmol^{-1}	$1{\cdot}12 \times 10^{6}$	$6{\cdot}77 \times 10^{6}$	$6{\cdot}48 \times 10^{6}$
Dilution	866	143	150
Incorporation/%	0·77[b]	0·54[b]	0·83[b]

[a] 83·3% uptake, feeding period 46 h, $9{\cdot}70 \times 10^{8}$ d.p.m. mmol^{-1}; administered $3{\cdot}58 \times 10^{7}$ d.p.m. [b] Corrected for uptake.



SCHEME. Biosynthesis of rotenoids in *A. fruticosa*.

in which *E*-[4'-^{3}H]rot-2-enonic acid was administered to 1200 *A. fruticosa* seedlings. The isolated dalpanol (**4**) had some six times the specific activity of amorphigenin (**6**), consistent with its position in the biosynthetic pathway.†

E-[4'-^{14}C]Rot-2'-enonic acid (**2**) (3·57 mg, 1·12 × 10^9 d.p.m. mmol^{-1}) containing ^{14}C-label distribution at C-4' (88%) and C-5' (12%)[2] was administered to 1000 *A. fruticosa* seedlings (uptake 69%). After 48 h, radiolabelled rotenone was isolated by addition of unlabelled rotenone and crystallised to constant activity (incorporation 1·10%). Degradation of a specimen (7·61 × 10^4 d.p.m. mmol^{-1}) by oxidative cleavage gave the nor-ketone (**8**) (1·37 × 10^4 d.p.m. mmol^{-1}) and formaldehyde as its dimedone derivative (5·60 × 10^4 d.p.m. mmol^{-1}). With 92% of the activity accounted for, the labelling pattern, after correction for ^{14}C-label located at C-5' in the original rot-2'-enonic acid, is 91·3% in the C-7'-methylene and 8·7% in the C-8'-methyl of rotenone. This 10·6 : 1 C-7' : C-8' ratio of labelling demonstrates high stereoselectivity in the overall conversion (**2**) → (**5**). Considering that rotenone occurs in limited amount and is not the main terminal metabolic product, and that a radioactive precursor with distributed labelling is employed, experimental error is bound to be moderately high. It seems very probable that the overall process is actually stereospecific, consisting of three stereospecific steps.

The first of these, (**2**) → (**3**) can lead to a 2'-*S*-3'-*pro*-*S*-epoxide as shown, or the diastereomeric 2'-*R*-3'-*pro*-*R*-epoxide. If the process (**3**) → (**4**) involves normal rearward attack on the epoxide, the 2'-*S*-3'-*pro*-*S* epoxide must be formed in order to generate the established β-orientation at C-5' of dalpanol and rotenone. The other diastereomer would give the unnatural α-orientation. This leads to a 5'-*R*-6'-*pro*-*S* configuration for dalpanol (**4**); dehydration involving the labelled 7'-*pro*-*S*-methyl then leads to rotenone (**5**), ultimately hydroxylated to amorphigenin (**6**).

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† 12aβ-Hydroxyamorphigenin (**10**) was also isolated and its high activity sheds light on the problem of 12a-hydroxyrotenoids as natural products. They are readily formed *in vitro* from rotenoids, especially under slightly basic conditions, by aerial oxidation, so that reports of their occurrence as true natural products are sometimes open to question. Since the specific activity of (**10**) is some six times greater than amorphigenin (**6**) it cannot have arisen from the latter as an artefact of isolation; its possible origins will be discussed later.

[1] L. Crombie, P. M. Dewick, and D. A. Whiting, *J.C.S. Perkin I*, 1973, 1285.
[2] L. Crombie, I. Holden, G. W. Kilbee, and D. A. Whiting, *J.C.S. Chem. Comm.*, 1979, preceding communication.