Hydroxylation of Rotenone to Amorphigenin in *Amorpha fruticosa* Seedlings

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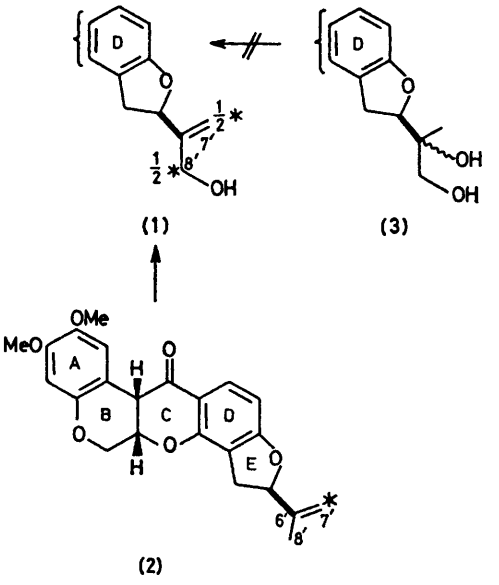
Summary Rotenone (**2**), but neither of the 6′-epimers of amorphigenol (**3**), is an excellent precursor for amorphigenin (**1**) in *Amorpha fruticosa*; using [7′-^{14}C]rotenone and three different work-up techniques, the ^{14}C-label was found to be equivalently distributed between the 7′-methylene and 8′-hydroxymethylene of amorphigenin.

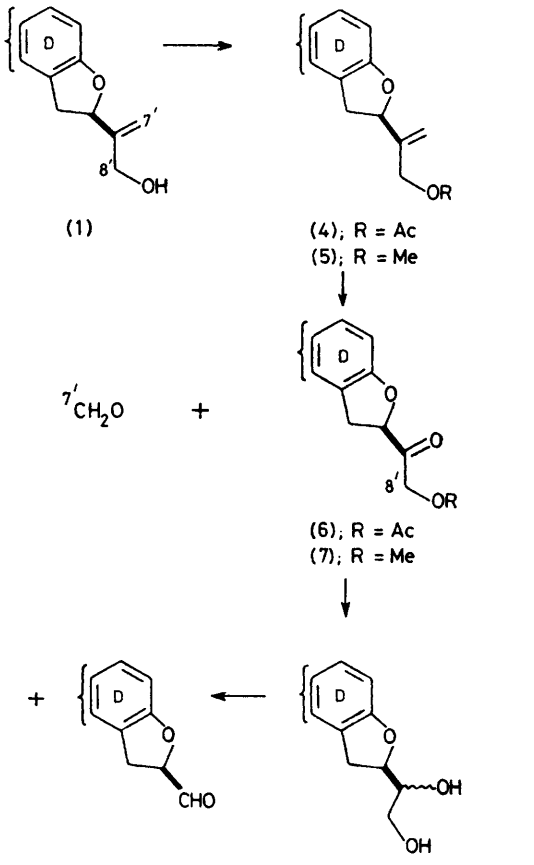
AMORPHIGENIN (**1**) is the major rotenoid metabolite of *Amorpha fruticosa* seedlings and our earlier work[1,2] indicates that it is formed *in vivo* by oxidation of rotenone (**2**). This oxidation might be a direct allylic oxidation, or proceed *via* amorphigenol (**3**).[3] Both (**2**) and (**3**) are present in the seedlings, the latter also occurring as a glycoside.



SCHEME 1. Rotenoid relationships in *A. fruticosa*.

In order to reach a decision between these two possibilities, [7′-^{14}C]rotenone was oxidised with *N*-methylmorpholine *N*-oxide and a catalytic quantity of osmium tetroxide[4] to give the pair of 6′-epimers (**3**) (3 : 1 by n.m.r. spectroscopy). Both [7′-^{14}C]rotenone (**2**) and the [7′-^{14}C]-6′α,6′β-amorphigenols were administered to *A. fruticosa* seedlings with the results shown in Table 1. Clearly rotenone is an excellent precursor, but although absorbed by the seedlings, neither the 6α- nor the 6β-epimer of (**3**) was metabolised to amorphigenin.

Preliminary experiments indicated that the [7′-^{14}C] label of rotenone became evenly distributed between the 7′-methylene and 8′-hydroxymethylene of amorphigenin and since these centres are part of an allylic system the possibility of equilibration during work-up or derivatisation was scrutinised. Amorphigenin (from Table 1) was isolated and degraded according to Scheme 2 in three separate experiments (Table 2), all activities being measured on solids recrystallised to constant activity. In experiment A, amorphigenin was isolated and purified by preparative layer chromatography (silica), acetylated (acetic anhydride–pyridine), and degraded. The figures show, within experi-



SCHEME 2. Degradation of amorphigenin (formaldehyde isolated as the dimedone derivative).

TABLE 1. Administration of ^{14}C-precursors to *A. fruticosa* seedlings (48 h): formation of amorphigenin (**1**).

	Total activity of precursor /d.p.m.	Incorporation[b] /%	Dilution	Uptake /%
[7′-^{14}C]Rotenone (**2**)	$1{\cdot}19 \times 10^7$	1·89	573	97
[7′-^{14}C]Rotenone (**2**)[a]	$2{\cdot}70 \times 10^7$	0·57	7960	96
[7′-^{14}C]-6′α- and -6′β-Amorphigenols (**3**)	$1{\cdot}50 \times 10^6$	0	—	69

[a] Amorphigenin from this experiment used in Table 2. [b] Corrected for uptake.

TABLE 2. Degradation of ^{14}C-labelled amorphigenin.

	Specific activity /d.p.m. mmol^{-1}	Relative activity
Experiment A		
(**1**)	$1{\cdot}31 \times 10^6$	1·00
(**4**)	$1{\cdot}31 \times 10^6$	1·00
Dimedone of 7′-formaldehyde	$6{\cdot}23 \times 10^5$	0·48
(**6**)	$6{\cdot}31 \times 10^5$	0·48
Dimedone of 8′-formaldehyde	$7{\cdot}06 \times 10^5$	0·54
Experiment B		
(**1**)	$1{\cdot}31 \times 10^6$	1·00
(**5**)	$1{\cdot}33 \times 10^6$	1·01
Dimedone of 7′-formaldehyde	$6{\cdot}16 \times 10^5$	0·47
Oxime of nor-ketone ether (**7**)	$6{\cdot}23 \times 10^5$	0·48
Experiment C		
(**5**)	$9{\cdot}67 \times 10^5$	1·00
Dimedone of 7′-formaldehyde	$4{\cdot}29 \times 10^5$	0·44
Oxime of nor-ketone ether (**7**)	$4{\cdot}62 \times 10^5$	0·48

mental error, equal isotope distribution between C-7′ and C-8′. To circumvent the possibility that acylation might be implicated in 7′,8′-scrambling the methyl ether (**5**) was formed instead ($MeI,Ag_2O,CHCl_3$) in low polarity medium (expt. B). In experiment C the amorphigenin was isolated by dilution and direct crystallisation, avoiding any adsorption on silica, and the methyl ether was degraded. In both experiments B and C the ^{14}C-label was again equally shared between C-7′ and C-8′.

Thus the label at C-7′ in rotenone becomes at some stage chemically equivalent to C-8′. Allylic rearrangement during extraction (95% aqueous ethanol) appears unlikely, particularly as there is no evidence of solvent participation; *i.e.*, no 8′-ethoxyrotenone is formed. *In vivo* possibilities remain. Biological hydroxylation of rotenone may involve a symmetrical allyl species, or there could be regiospecific hydroxylation of rotenone followed by subsequent *in vivo* scrambling. The latter possibility is not ruled out since amorphigenin occurs both free and as the vicianoside (and perhaps other bound forms), and aglycone–glycoside equilibration probably occurs.

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