# Biosynthetic Studies of Ipomeamarone

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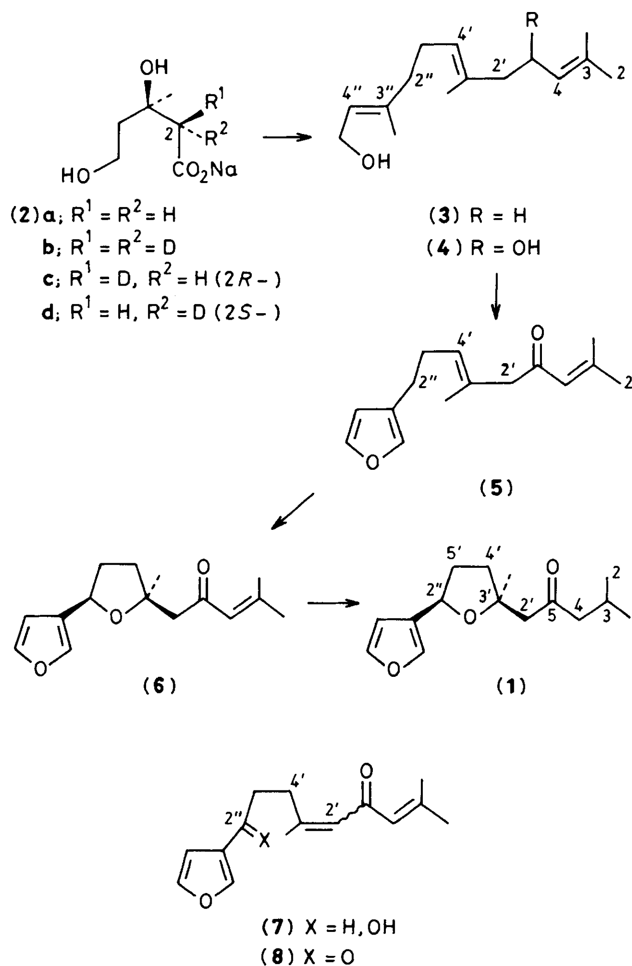
Incorporation studies of deuteriomevalonates (**2b—d**) into ipomeamarone (**1**) have shown that an unusual double bond migration involving stereospecific 1,3-H-transfer takes place.

The furanoterpene, ipomeamarone (**1**)[1a—c] is the major phytoalexin produced by sweet potato (*Ipomea batatas*) upon infection with pathogens such as *Ceratocystis fimbriata*. The sesquiterpenoid nature of (**1**) was established by incorporation of radiolabelled acetate and mevalonate (MVA) (Scheme 1).[2] However, although the incorporation of farnesol (**3**),[3] 9-hydroxyfarnesol (**4**), 6-oxodendrolasin (**5**),[4] and dehydro-ipomeamarone (**6**)[5] into (**1**) has been reported, the biogenetic route of this important class of phytoalexin is poorly defined. In the following we have carried out incorporation studies with deuteriated mevalonates and have shown the occurrence of an unusual stereospecific 1,3-migration of hydrogen from C-2′† to C-4′.

The most direct pathway to (**6**) involves C-2″ hydroxylation of (**5**) followed by acid-catalysed cyclization. Alternatively, C-2″ hydroxylation and double bond isomerization gives (**7**), which upon Michael-type cyclization would furnish (**6**).[4] On the other hand, we have recently isolated 1,6-dioxo-isodendrolasin (**8**),[6] which could be reduced to give (**7**) [and then (**6**)]. To determine which of these pathways, if any, is operative, we have transformed intermediate [2,2′,2″-$^2H_6$]-(**5**), which in turn was produced from [2,2-$^2H_2$]MVA, into (**1**).

Incubation[7] of 1.2 g of (±)-[2,2-$^2H_2$]MVA (**2b**) with 100 g of *C. fimbriata*-infected sweet potato gave 98 mg of deuteriated (**1**) [3.9% incorporation based on 3*R*-(**2b**)]. The molecular ion in the mass spectrum of (**1**) indicated that five deuterium atoms had been incorporated.‡ We can thus exclude all intermediates with a C-2″ carbonyl group since only four deuterons would have been retained in that case.

The $^{13}C$ n.m.r. spectrum of deuteriated (**1**) showed that the C-2″ peak at δ 72.6 p.p.m. and C-2 peak at δ 22.5 p.p.m. were




**Scheme 1.** Biosynthetic precursors of ipomeamarone (**1**). The numbering system follows that of the mevalonate unit.

† For the sake of clarity, numbering of the ipomeamarone skeleton is based on that of the mevalonate unit.

‡ Mass spectral data (70 eV): *m/z* 250 ($M^+$, 100%), 251 ($M^+$ + 1, 45%), 252 ($M^+$ + 2, 35%), 253 ($M^+$ + 3, 27%), 254 ($M^+$ + 4, 19%), 255 ($M^+$ + 5, 8%).

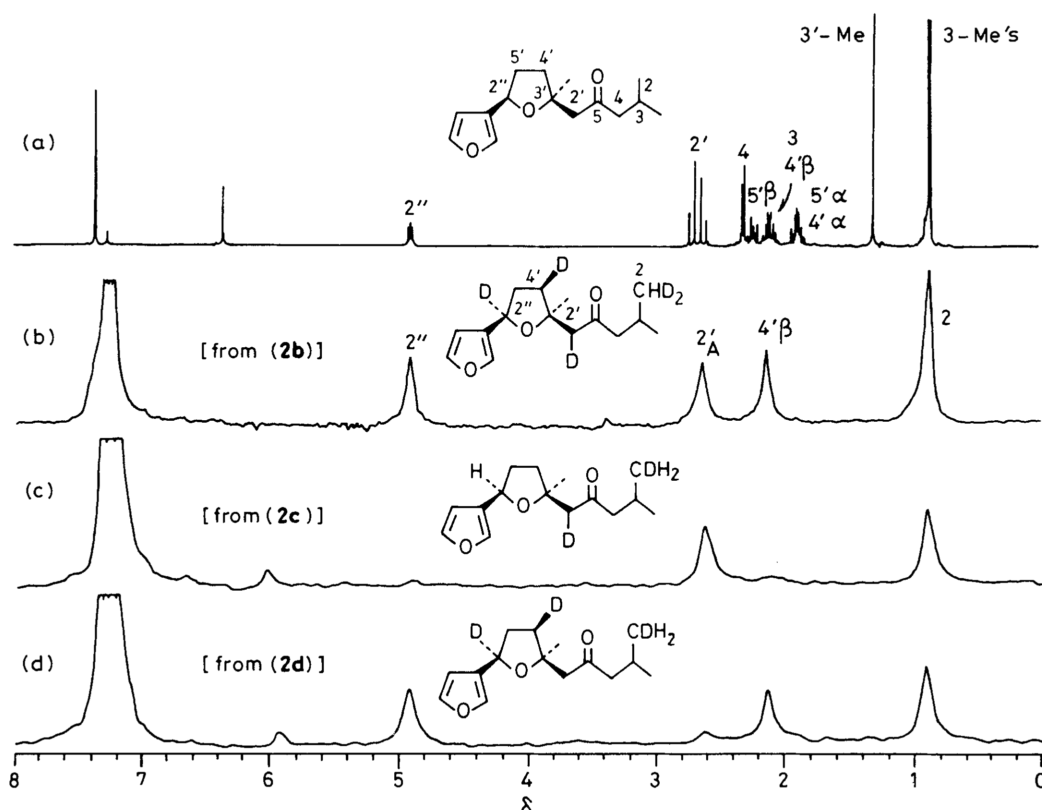


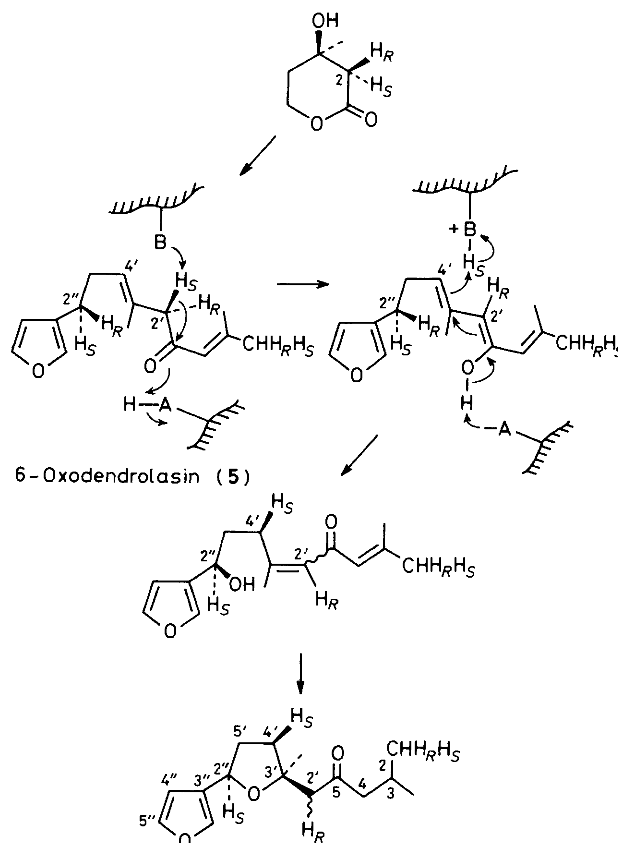

**Figure 1.** (a) Unlabelled ipomeamarone (**1**), ¹H n.m.r. spectrum (360 MHz, $CDCl_3$); (b) labelled (**1**) from [2-$^2H_2$]MVA (**2b**), ²H n.m.r. spectrum (55.27 MHz, 3% $CDCl_3$ in $CHCl_3$); (c) labelled (**1**) from 2*R*-[2-$^2H_1$]MVA (**2c**); (d) labelled (**1**) from 2*S*-[$^2H_1$]MVA (**2d**). Note that the 2'-H signals in (**1**) derived from (**2b**) and (**2c**) correspond to the higher field doublet of the ABq in (**1**) derived from (**2a**).

accompanied, respectively, by an upfield triplet (δ 72.7 p.p.m., *J* 23 Hz, C-D) and a quintet (δ 21.8 p.p.m., *J* 20 Hz, $CD_2H$). Surprisingly, however, C-2' (δ 54.2 p.p.m.) which should have been labelled with two deuterons, showed the presence of only one deuteron (triplet at δ 53.9 p.p.m., *J* 21 Hz), whereas the C-4' signal (δ 37.0 p.p.m.) was accompanied by a δ 36.6 p.p.m. triplet, *J* 21 Hz. These results indicate the migration of one hydrogen from C-2' to C-4' during the tetrahydrofuran biogenesis. Furthermore, the ²H n.m.r. spectrum§ revealed that deuteriations at C-4' and C-2' are both stereochemically homogeneous [Figure 1(b)]. Since the integrated intensity ratio of 2"-, $2'_A$-,¶ 4'β-, 2-²H (*i.e.*, 3-Me) peaks was 1 : 1 : 1 : 2 (±5%) we conclude that a *stereospecific 1,3-hydrogen(deuterium) transfer from C-2' to C-4' had occurred with complete retention of deuterium.*

Usual enzymatic double bond migrations result either in the incorporation of hydrogen from the medium,[8,9] or in varying degrees of hydrogen loss in the case of an allylic transfer.[10,11] In order to identify the hydrogen which migrates from C-2' to C-4', labelled mevalonates 2*R*-[2-$^2H_1$]MVA (**2c**) (1.1 g) and 2*S*-[2-$^2H_1$]MVA (**2d**)[12] (1.0 g) were incubated with infected sweet potato root tissue to yield, respectively, 100 mg of

§ See Figure 1. The ¹H n.m.r. δ values of 5'β (2.22), 3/4'β (*ca.* 2.1), and 5'α/4'α protons (*ca.* 1.9) were assigned as follows: (i) irradiation of 2"α-H (δ 4.86) decoupled the δ 2.22 (5'β) and 1.9 (5'α/4'α) signals; (ii) irradiation of 3'-methyl (δ 1.33) induced nuclear Overhauser enhancements on the δ 1.9 signals (5'α/4'α) besides the δ 4.86 signal (2"α) and not on the δ 2.1 signals (3/4'β).

¶ Both ¹H and ²H n.m.r. spectra indicated that only half of the C-2' AB quartet (δ 2.55) was deuteriated. However, the stereochemistry at C-2' remains unknown as we were unable to assign the two halves of the AB quartet to specific protons.




**Scheme 2.** Schematic representation of ipomeamarone biosynthesis; *R* and *S* represent the prochirality of the 2-H's in mevalonate.

$[2,2'-{}^2H_2]$-(**1**) [Figure 1(c)] and 30 mg of $[2,4',2''-{}^2H_3]$-(**1**) [Figure 1(d)]. The $^2H$ n.m.r. proves that the $4'\beta-{}^2H$ of (**1**) [Figure 1(d)] originates from $2S-{}^2H$ of mevalonate (**2d**); as shown in Scheme 2, this hydrogen is attached to C-2′ in intermediate (**5**). We have thus shown that a stereospecific 1,3-hydrogen shift occurs in the biosynthesis of (**1**). The lack of proton exchange during this process can be accounted for by a mechanism such as that shown in Scheme 2, in which the same basic group B of an enzyme is involved in deprotonation at C-2′ and reprotonation at C-4′. This appears to be the first example of a stereospecific allylic isomerization[13] which occurs with complete retention of hydrogen.

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