# The Biosynthesis of 2,7-Dihydroxycadalene in Infected Cotton Cotyledons: The Folding Pattern of the Farnesol Precursor and Possible Implications for Gossypol Biosynthesis

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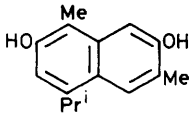
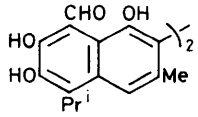
Incorporation of [1,2-$^{13}C_2$]acetate into the cotton phytoalexin 2,7-dihydroxycadalene by bacteria-inoculated cotton seedlings has established a folding pattern for its carbon skeleton which differs from that deduced for the other cotton phytoalexin gossypol by earlier $^{14}C$ studies.

2,7-Dihydroxycadalene (**1**)[1,2] is one of the metabolites whose production is stimulated in leaves and cotyledons of bacterial blight-resistant cotton after inoculation with the pathogen *Xanthomonas campestris* pv. *malvacearum*.[1] The compound is toxic to the bacterium and is believed to be partly responsible for the subsequent inhibition of its growth in the plant.[1] It has the same cadinane carbon skeleton as the other cotton phytoalexin gossypol (**2**), whose biosynthesis has been investigated by Heinstein *et al.* [3,4]

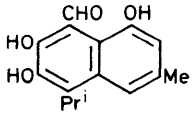
To study the biosynthesis of (**1**), cotyledons of 12 day-old cotton seedlings (line OK 1.2) raised in a growth chamber were infiltrated with a suspension of the bacterium[1] and then with a solution of [1,2-$^{13}C_2$]acetate (90% $^{13}C$; 70 ml, 20 mM, applied in equal amounts to 57 seedlings) at the predetermined time of incipient maximal cadalene synthesis (52 h after inoculation). When concentrations of (**1**) had reached a peak (70 h), as determined in a preliminary experiment, the cotyledons were harvested and extracted essentially as previously described (method 2[1]). 2,7-Dihydroxycadalene (**1**) was



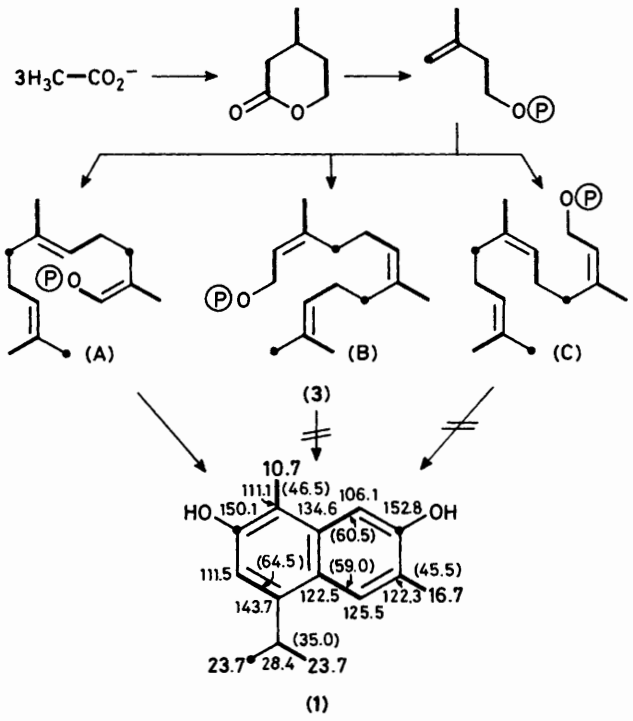

(1)




(2)




(4)




**Scheme 1.** Biosynthetic folding pattern, $^{13}C$ shieldings, and observed couplings (in parentheses) for (**1**).

isolated from the product by h.p.l.c. (octadecylsilane; MeOH–$H_2O$, 62:38 v/v) as a gum (*ca.* 1.4 mg) which was better than 95% pure by t.l.c. and the $^1H$ n.m.r. spectrum. The $^{13}C$ n.m.r. spectrum of the sample indicated an enrichment of *ca.* 3% at all carbons.

The most striking feature of the $^{13}C$ spectrum is the fact that the signals for the two oxygen-bearing carbons, readily identified by their appearance near $\delta_C$ 150,[5] were singlets while the other eight aryl carbons exhibited the characteristic satellites arising from $^{13}C$–$^{13}C$ coupling between adjacent labelled centres. These satellites were also evident for the four aliphatic patterns with the one important difference that the central signal for the two equivalent isopropyl methyl carbons ($\delta_C$ 23.7) was *ca.* four times as intense as the other methyl peaks, clearly showing that only one of the isopropyl methyl carbons arises from an intact acetate unit. The higher field methyl patterns ($\delta_C$ 10.7 and 16.7) were readily assigned to the 1- and 6-methyl carbons, respectively, since the former is in the *peri* position and will experience greater shielding. The coupling constants for each served to identify C-1 and C-6. Of the three protonated aryl carbons, C-3 and C-8 are significantly shielded by the *ortho* hydroxy groups[5] and, for C-8, the additional shielding of the *peri*-methyl group; their couplings identified C-4 and C-8a. The common couplings exhibited by the two remaining patterns distinguished C-4a and C-5. The $^{13}C$ shieldings and the observed couplings (in parentheses) for the carbons incorporated as intact doubly-labelled acetate units are shown in (**1**) (Scheme 1). These data establish that farnesyl pyrophosphate (**3**), or equivalent precursor, cyclizes to (**1**) from the folding pattern (A) and not the alternatives (B) or (C) (see Scheme 1), since only (A) will lead to the observed singlets for both C-2 and C-7. The labelling patterns found for the remaining carbons are in complete agreement.

The mode of cyclization thus ascertained for (**1**) does not correspond to the results of Heinstein *et al.*[3,4] for gossypol (**2**) [formed by the oxidative dimerization of hemigossypol[6] (**4**)]. Using $^{14}C$-labelled precursors, these workers concluded, on the basis of degradation experiments, that (**2**) was labelled in a manner corresponding to folding pattern (C). It is conceivable that (**4**), and hence (**2**) arise from a different pattern than that for the biosynthesis of (**1**), particularly since the studies by Heinstein *et al.*,[3] in contrast to our own, were conducted with cell-free enzyme preparations. Nevertheless, because of the very close relationship between the compounds, a re-examination of gossypol biosynthesis, using different conditions, would now seem advisable.

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