Biosynthesis of Helicobasidin and Related Compounds

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Summary During the biosynthesis of helicobasidin (possessing a cuparane skeleton) hydride shifts have been shown to occur, excluding a possibility of the intermediacy of γ-bisabolene.

HELICOBASIDIN (I) is a fungal pigment produced by *Helicobasidium mompa* Tanaka (Tremellales, Basidiomycetes), a plant pathogenic fungus causing violet root rot disease.[1] The isoprenoid origin of helicobasidin has been proved and its biogenesis discussed.[2,3]

In connection with studies of the mechanism of enzymic cyclisation of the acyclic isoprenyl precursor, we have studied the biosynthesis of helicobasidin and related compounds using doubly labelled mevalonic acid lactone as a substrate.

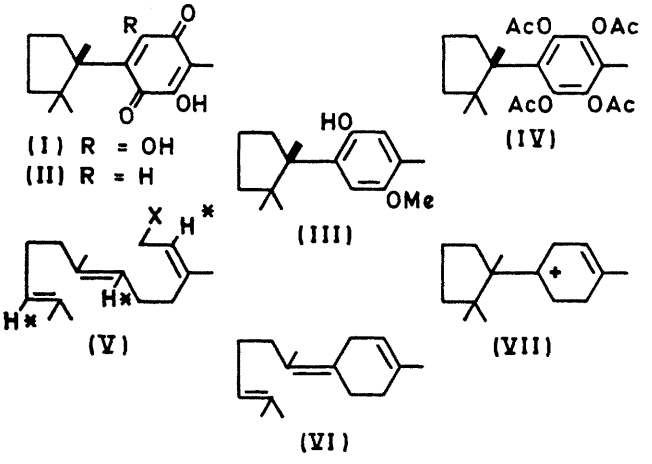
[2-^{14}C-4R,4-^{3}H]Mevalonic acid lactone was fed to *Helicobasidium mompa*. After 14 days the culture was harvested and the mycelia and the filtrate were extracted with organic solvent. The products were then separated by silica gel column chromatography into four fractions, containing, respectively, helicobasidin (I), deoxyhelicobasidin (II),[2] the compound (III),[4] and ergosterol. The biosynthesised helicobasidin (I) was reduced, after dilution by a pure sample, with zinc in acetic anhydride to give a leuco-acetate (IV).[2] The compounds (II), (III), (IV), and ergosterol were crystallised to constant specific activity after the addition of carrier substances. The ^{3}H : ^{14}C ratios for each compound are given in the Table. Ergosterol was expected to have a ratio of 3 : 5 according to the established biosynthetic mechanism.[5]

Observed ^{3}H/^{14}C ratios of biosynthetic compounds

Compound	Incorp. (%)	^{3}H/^{14}C (d.p.m.)	^{3}H/^{14}C (atomic)
Mevalonic acid lactone ..	—	6·46	3 : 3
Compound (III)	0·6	6·43	2·99 : 3
Leuco-acetate (IV)	1·7[a]	3·99	1·85 : 3
Deoxyhelicobasidin[b] (II) ..	0·7	4·84	2·20 : 3
Ergosterol	0·2	3·96	3·07 : 5

[a] Incorporation for helicobasidin (I).
[b] Leuco-acetate was used for counting of radioactivity.

These results provide evidence that all the *pro-R*-hydrogen atoms from C-4 in mevalonic acid, which were known to be located on the double bonds in the farnesyl precursor[6] (H asterisked in V), are retained in the cuparene derivative (III), and two of them might be located on the five-membered ring [from the data on the leuco-acetate (IV)]. This finding may exclude, at least in micro-organisms, the intermediary role of γ-bisabolene (VI)[7,8] for the formation of the cuparane skeleton. Although the true biosynthetic cyclisation mechanism is still unknown,[9] one possibility is that the ion (VII) is formed directly from an acyclic precursor (*e.g.*, V) by solvolytic cyclisation followed by hydride shifts.



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