Studies in Terpenoid Biosynthesis. Part VII.[1] The Biosynthesis of Helicobasidin

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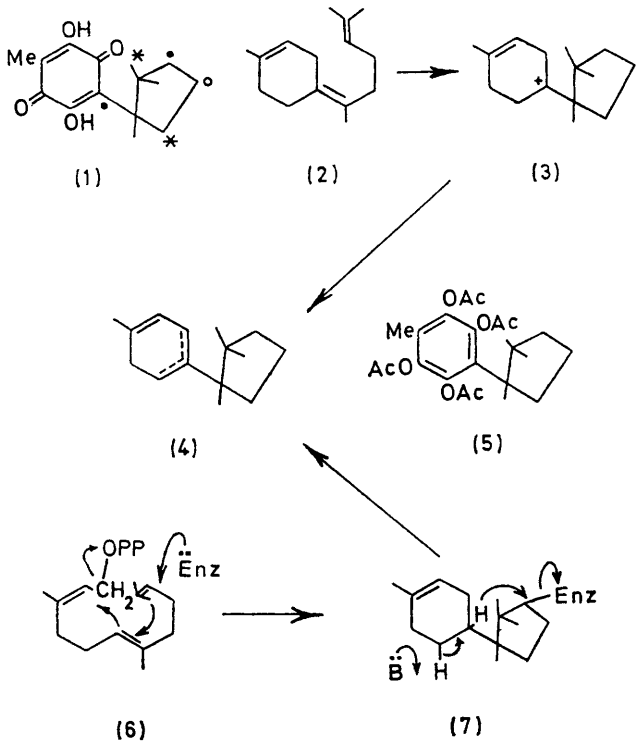
Helicobasidin has been shown to incorporate four tritium atoms from [2,2-$^3H_2$]mevalonate, two from 4*R*-[4-^{3}H]-mevalonate, and two from [5,5-$^3H_2$]mevalonate. Feeding with [2-^{3}H,2-^{14}C]geranyl pyrophosphate has shown that the 4*R*-mevalonoid tritium atom from the second prenyl unit is retained in the biosynthesis.

THE fungal pigment helicobasidin (1), isolated from *Helicobasidium mompa*,[2] has been shown to be sesquiterpenoid.[3,4] On biogenetic grounds it has been suggested [5] that helicobasidin belongs to the cuparane class, whose carbon skeleton is thought to arise [6] by protonation of γ-bisabolene (2) followed by cyclization to the tertiary cation (3) which could then afford the cuprenenes (4). In view of the close relationship of this scheme to that which has been proposed for the tricothecanes (*cf.* ref. 7), we have examined the origin of the skeletal hydrogen atoms of helicobasidin. If either the bisabolene route or the 'direct' theory of Bentley and Chen [4] is correct then helicobasidin should incorporate up to four tritium atoms from the [2,2-$^3H_2$]mevalon-

[1] Part VI, R. Evans, J. R. Hanson, and A. F. White, *J. Chem. Soc.* (*C*), 1970, 2601.
[2] S. Natori, H. Nishikawa, and H. Ogawa, *Chem. and Pharm. Bull.* (*Japan*), 1974, **12**, 236.
[3] S. Natori, Y. Inouye, and H. Nishikawa, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 380.
[4] R. Bentley and D. Chen, *Phytochemistry*, 1969, **8**, 2171.
[5] W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, 1967, **21**, 331.
[6] C. Enzell and H. Erdtman, *Tetrahedron*, 1958, **4**, 361.
[7] P. M. Adams and J. R. Hanson, *Chem. Comm.*, 1970, 1569.

ate progenitor, one from 4*R*-[4-^{3}H]mevalonate, and two from [5,5-$^3H_2$]mevalonate. While this work was in progress, a note appeared [8] which, on the basis of the incorporation of two 4(*R*)-mevalonoid protons, excluded



* Label from 2-position in mevalonic acid; ●, from 4-position; and ○, from 5-position. PP = Pyrophosphate.

a γ-bisabolene intermediate, and proposed a direct cyclization to a cuparenyl cation. Our results also exclude the bisabolene pathway.

[2,2-$^3H_2$,2-^{14}C]Mevalonic acid lactone (^{3}H : ^{14}C, 7·56 : 1) was fed to *Helicobasidium mompa* 70 days after inoculation. After a further 13 days the helicobasidin was isolated from the mycelium by sublimation. To overcome quenching problems, the helicobasidin was converted into its leuco-acetate (5) by reduction with zinc and acetic anhydride. This showed activity (^{3}H : ^{14}C, 4·92 : 1) corresponding to 3·9 tritium atoms. When 4*R*-[4-^{3}H,2-^{14}C]mevalonic acid lactone (^{3}H : ^{14}C, 9·33 : 1) was used, surprisingly, the helicobasidin leuco-acetate showed an activity (^{3}H : ^{14}C, 5·74 : 1) corresponding to the retention of two tritium atoms, in agreement with the results of Nozoe.[8] Finally when [5,5-$^3H_2$,2-^{14}C]-mevalonic acid, as its *NN'*-dibenzylethylenediamine salt (^{3}H : ^{14}C,10·4 : 1), was used, the helicobasidin leucoacetate showed an activity (^{3}H : ^{14}C, 3·3 : 1) corresponding to the incorporation of 1·9 mevalonoid tritium atoms.

The origin of the additional 4-*pro*-*R*-mevalonoid proton from the central prenyl unit of farnesyl pyrophosphate precursor (6) [*i.e.*, from C-6 of the pyrophosphate (6)] was established by feeding experiments with [2-^{3}H,2-^{14}C]geranyl pyrophosphate (^{3}H : ^{14}C, 7·06 : 1). The helicobasidin leuco-acetate showed the relative activity (^{3}H : ^{14}C of 7·04 : 1) and thus the 4-*pro*-*R*-mevalonoid proton from the central prenyl unit has been retained in the biosynthesis.

If we make the reasonable assumption that the mevalonoid labels are not 'scrambled' during the biosynthesis, then these results exclude γ-bisabolene as an intermediate in the fungal pathway. The location of the labels in the related tricothecane case has been established.[7,9] Furthermore the retention of the central prenyl olefinic proton, which in the tricothecane case has been shown to occupy the 2-position,[9] suggests that a direct cyclization of farnesyl pyrophosphate (6) occurs. The primary enzyme-bound intermediate (7) may be displaced from the enzyme surface by a hydride shift and elimination which leads to the cuprenenes (4) and thence by oxidation to deoxyhelicobasidin and helicobasidin.

EXPERIMENTAL

General details have been described previously.[10] Counting was carried out with a Beckman LS 100 liquid scintillation counter. *Helicobasidium mompa* was grown as a still culture in Roux bottles (250 ml) and in Thompson bottles (750 ml) on a medium containing (per litre) sucrose (20·0 g), potassium dihydrogen phosphate (0·5 g), dipotassium hydrogen phosphate (0·5 g), magnesium sulphate (0·2 g), manganese sulphate (0·01 g), ferrous sulphate (0·01 g), sodium chloride (0·01 g), and L-asparagine (3·0 g). The ferments were harvested 80—90 days after inoculation. The helicobasidin was recovered simply from the dried mycelium by sublimation. The mycelium (95 g) from 10 l afforded helicobasidin (250 mg) as orange needles, m.p. 194° (from ethanol), $[\alpha]_D^{20}$ −125° (*c* 0·1 in $CHCl_3$) {lit.,[2,3] 190—192°, $[\alpha]_D^{25}$ −123° ($CHCl_3$)} (Found: C, 68·1; H, 7·5. Calc. for $C_{15}H_{20}O_4$: C, 68·2; H, 7·6%), τ 9·17 (3H, s), 8·93 (3H, s), 8·67 (3H, s), and 8·08 (3H, s). The fermentations also yielded mompain and deoxyhelicobasidin.

Incorporation of [2,2-$^3H_2$,2-^{14}C]*Mevalonic Acid Lactone.*—70 Days after inoculation, the mevalonate (^{3}H : ^{14}C, 7·56 : 1) in ethanol (15 ml) was distributed in *Helicobasidium mompa* (3·5 l). The ferment was harvested after a further 14 days and the active helicobasidin (25 mg), m.p. 194°, was isolated and diluted with inactive material (35 mg). It was heated in acetic anhydride (4 ml) under reflux with zinc powder (400 mg) for 2 h. The hot filtrate was poured into water and neutralized with sodium carbonate. Extraction with ether led to helicobasidin leuco-acetate, white prisms (30 mg), m.p. 156—158° (from ethyl acetate–light petroleum), $[\alpha]_D^{20}$ −9·8° (*c* 0·3 in $CHCl_3$) {lit.,[3] 152—154°, $[\alpha]_D^{25}$ −9·5° ($CHCl_3$)} (Found: C, 63·9; H, 7·15. Calc. for $C_{23}H_{30}O_8$: C, 63·6; H, 7·0%), τ 9·23 (3H, s), 8·98 (3H, s), 8·72 (3H, s), 8·06 (3H, s), and 7·74 (12H, s), relative activity ^{3}H : ^{14}C, 4·92 : 1).

Incorporation of 4R-[4-^{3}H,2-^{14}C]*Mevalonic Acid Lactone.*—70 Days after inoculation, the (^{3}H : ^{14}C, 9·33 : 1) in

[8] S. Nozoe, M. Morisaki, and H. Matsumoto, *Chem. Comm.*, 1970, 926.

[9] P. M. Adams and J. R. Hanson, unpublished work.

[10] B. Achilladelis and J. R. Hanson, *Phytochemistry*, 1968, **7**, 589.

aqueous ethanol (1·75 ml) was distributed in *Helicobasidium mompa* (4 l). The ferment was harvested after a further 14 days and helicobasidin (44 mg), m.p. 193—194°, was recovered by sublimation. This was converted into the leuco-acetate (36 mg) (^3H : ^{14}C, 5·74 : 1), m.p. 156—158°, as before.

Incorporation of [5,5-$^3H_2$,2-^{14}C]*Mevalonic Acid.*—The mevalonate, as its *NN'*-dibenzylethylenediamine salt (^3H : ^{14}C, 10·4 : 1), in ethanol (2 ml) was distributed in *Helicobasidium mompa* (5 l) 74 days after inoculation. The ferment was harvested after a further 13 days and helicobasidin (10 mg), m.p. 193°, was isolated by sublimation. It was diluted with inactive helicobasidin (17 mg) and converted into its leuco-acetate (13 mg) (^3H : ^{14}C, 3·3 : 1), m.p. 158°, as before.

Incorporation of [2-^{3}H,2-^{14}C]*Geranyl Pyrophosphate.*—The pyrophosphate (^3H : ^{14}C, 7·06 : 1) in aqueous ethanol (12 ml) was distributed in *Helicobasidium mompa* (8 l) 70 days after inoculation. After a further 14 days the mycelium was filtered off, dried, and extracted with light petroleum. The extract (0·186 g) was purified by p.l.c. on silica gel which had been treated with 3% oxalic acid in water. Benzene–light petroleum (1 : 1) was used as the mobile phase. This gave helicobasidin (34 mg) (R_F 0·9), m.p. 188—191°, which was converted into the leuco-acetate (15 mg) (^3H : ^{14}C, 7·04 : 1), m.p. 157—159°, as before.

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