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HELICOBASIDIN: A FUNGAL BENZOQUINONE OF ISOPRENOID ORIGIN

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Abstract—Acetate-1-^{14}C and (±)-mevalonate-2-^{14}C were used as precursors for the biosynthesis of helicobasidin by *Helicobasidium mompa*. Degradation of the radioactive metabolite gave labelling patterns consistent with the origin of the cyclopentanoid and benzoquinoid rings from farnesyl pyrophosphate. Possible biosynthetic intermediates are considered.

INTRODUCTION

A FUNGAL benzoquinone, with an apparent structural relationship to the sesquiterpenes, is helicobasidin (I). This quinone was described as a product of Japanese strains of *Helicobasidium mompa* Tanaka[1] and its structure was elucidated by Natori *et al.*[2,3] From *H. mompa* Tanaka ATCC 11046 we have also isolated helicobasidin and have studied its biosynthesis from acetate-1-^{14}C and mevalonate-2-^{14}C. Chemical degradation of labelled helicobasidin supports an isoprenoid origin for this benzoquinone.

RESULTS AND DISCUSSION

The incorporations of radioactivity, achieved with these two precursors, are shown in Table 1. In view of the slow metabolic rate of this organism, the 0·9 per cent incorporation of activity from acetate-1-^{14}C is gratifying. A much lower incorporation was achieved with (±)-mevalonate-2-^{14}C. If only one enantiomer of this material was utilized, the incorporation was 0·4 per cent under conditions identical with those of the acetate experiment.

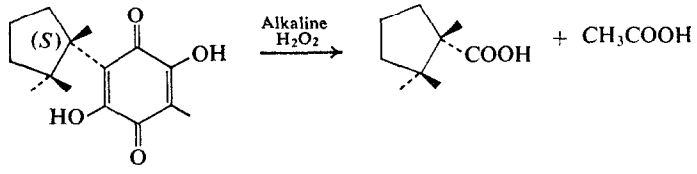
TABLE 1. INCORPORATION OF RADIOACTIVITY INTO HELICOBASIDIN

Labelled precursor	Amount added and time of addition	Time of harvest (days)	Helicobasidin, specific activity (dpm/mmole)	Activity incorporated (%)
Acetate-1-^{14}C	60 μc at day 73, 60 μc at day 76	80	$39{\cdot}6 \times 10^4$	0·9
(±)-Mevalonate-2-^{14}C	42 μc at day 73, 42 μc at day 76	80	$9{\cdot}7 \times 10^4$	0·04*

*Assuming that only the (*R*) enantiomer was utilized by the fungus.

[1] H. NISHIKAWA, *Agr. Biol. Chem.* **26**, 696 (1962).
[2] S. NATORI, H. OGAWA, K. YAMAGUCHI and H. NISHIKAWA, *Chem. Pharm. Bull.* **11**, 1343 (1963).
[3] S. NATORI, H. NISHIKAWA and H. OGAWA, *Chem. Pharm. Bull.* **12**, 236 (1964).

To locate radioactivity, samples of helicobasidin (I) were oxidized with alkaline H_2O_2.[3] The (−)-camphonanic acid (II) obtained in this oxidation was isolated and purified by TLC. Acetic acid, also produced in the oxidation, was recovered as its *p*-bromophenacyl ester. The distributions of radioactivity in the degradation products are shown in Table 2. In order to evaluate these labelling patterns, the fifteen carbon atoms of helicobasidin will be assumed to be derived from farnesyl pyrophosphate,[4–7] and three possible biosynthetic hypotheses will be considered.



(I) (II)

TABLE 2. DEGRADATION OF LABELLED HELICOBASIDIN SAMPLES

Compound	Acetate-1-^{14}C as precursor		(±)-Mevalonate-2-^{14}C as precursor	
	Specific activity (dpm/μmole)	Per cent of helicobasidin activity	Specific activity (dpm/μmole)	Per cent of helicobasidin activity
Helicobasidin	151	100	98	100
Camphonanic acid	72	48	66	67
Acetic acid (as *p*-bromophenacyl ester)	23	15	0	0

A. The Bisabolene Hypothesis

The involvement of γ-bisabolene (III), cuprenene (one isomer = IV), cuparene (V), and deoxyhelicobasidin (VI)* was suggested by Natori *et al.*, without taking into account certain stereochemical consequences.[3] The simplest cyclization of *trans–cis*–farnesyl pyrophosphate† (VIIa) yields the bisabolene (IIIa), and similarly *cis–cis*–farnesyl pyrophosphate (VIIb) yields the bisabolene (IIIb). Moreover, by further introduction of well-recognized rearrangements, *both* bisabolenes may be derived from either isomer of farnesyl pyrophosphate.[4]

If the further intermediates did not allow a randomization of the labelled position in the six-membered ring, mevalonate-2-^{14}C could give rise to one of the labelled helicobasidins (Ia)

* Deoxyhelicobasidin (VI) has actually been isolated as a contaminant of helicobasidin.[3]

† The double bonds may be specified without ambiguity using the *Chemical Abstracts E* and *Z* nomenclature.[8] Thus, in Δ^6-*trans*-Δ^2-*cis*-farnesyl pyrophosphate the Δ^6 bond is *E* and the Δ^2 bond is *Z*. In the Δ^6-*cis*-Δ^2-*cis* isomer, both double bonds are *Z*.

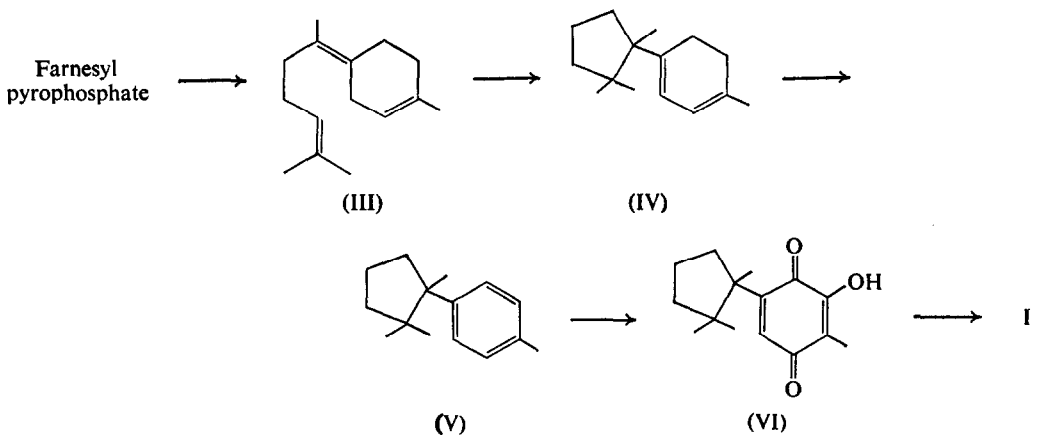
[4] L. RUZICKA, in *The Chemistry of Natural Products*, Vol. 2, p. 493, Butterworths, London (1963); reprinted from *Pure App. Chem.* **6**, 493 (1963).

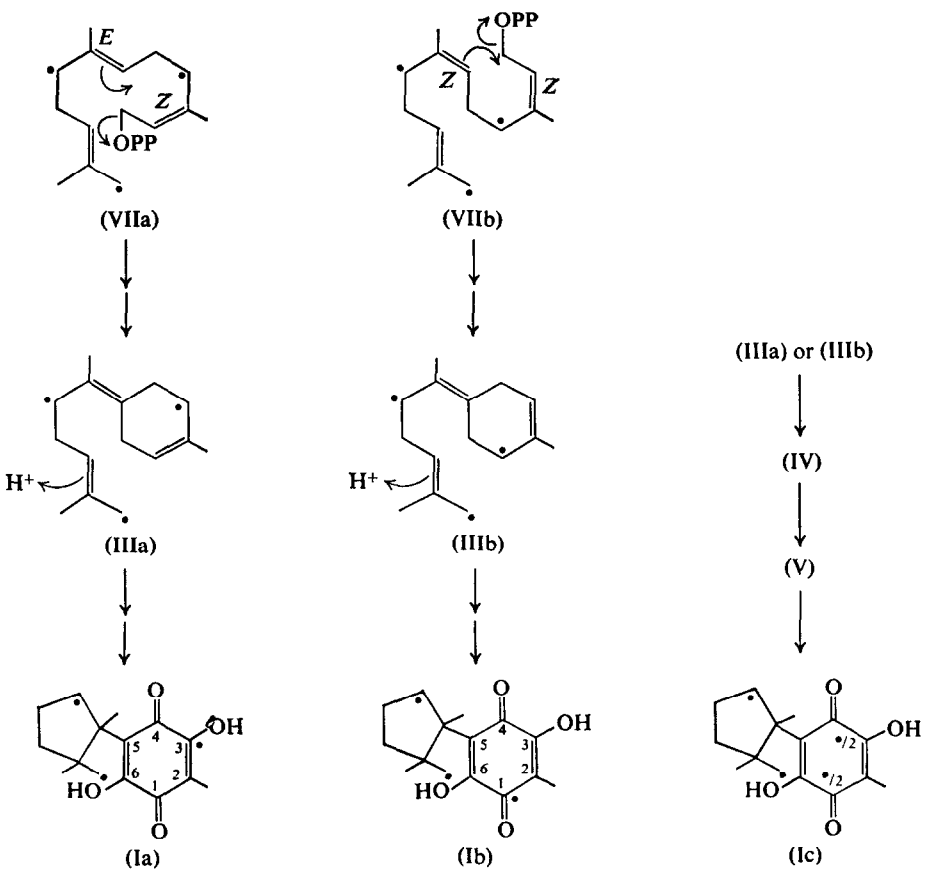
[5] J. B. HENDRICKSON, *Tetrahedron* **7**, 82 (1959).

[6] W. PARKER, J. S. ROBERTS and R. RAMAGE, *Quart. Rev.* **21**, 331 (1967).

[7] B. ACHILLADELIS and J. R. HANSON, *Phytochem.* **7**, 589 (1968).

[8] J. E. BLACKWOOD, C. L. GLADYS, K. L. LOENING, A. E. PETRARCA and J. E. RUSH, *J. Am. Chem. Soc.* **90**, 509 (1967).

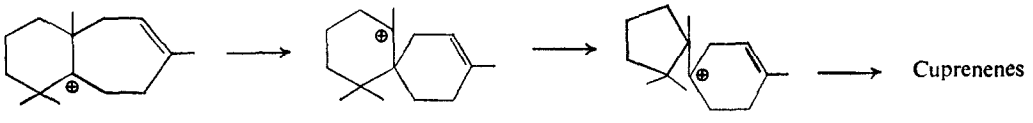

Farnesyl
pyrophosphate
(III)
(IV)
(V)
(VI)
O
OH
O
I


E
Z
OPP
(VIIa)
OPP
Z
Z
(VIIb)
H+
(IIIa)
H+
(IIIb)
(IIIa) or (IIIb)
(IV)
(V)
O
OH
HO
O
(Ia)
(Ib)
(Ic)

or (Ib), or to a mixture of both.* However, the postulated intermediate, cuparene, would lead to randomization between the positions arbitrarily numbered in (Ia) and (Ib) as 1 and 3 (and between 4 and 6). Thus, another possible labelling pattern in helicobasidin is that represented by (Ic); the symbol •/2 indicates half of the specific activity of •.

B. The Bicyclic Cation Hypothesis

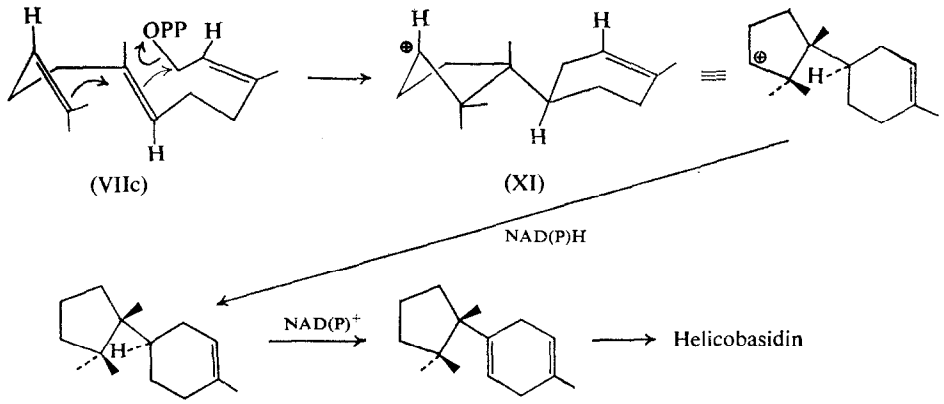
Dauben and Oberhänsli isolated from *Thujopsis dolabrata* oil, not only cuparene and two of the dextrorotatory cuprenenes, but also thujopsene and widdrol.[11] As a result, they suggested the cation (VIII) as a possible common precursor to all of these compounds; a cuprenene precursor (X) is obtained by rearrangement of (VIII) and (IX). Helicobasidin derived in this way from mevalonate-2-^{14}C would have the labelling pattern (Ib) or, if an intermediate allowing randomization were involved, (Ic).



(VIII) (IX) (X)

C. The Direct Concerted Cyclization Hypothesis

We postulate a direct concerted cyclization of farnesyl pyrophosphate as the mechanism for helicobasidin biosynthesis. Although models suggest considerable difficulty in forming the required six-membered ring if the Δ^2 bond of farnesyl pyrophosphate has a *trans* configuration, an easy cyclization (VIIc→XI) is possible if this bond is *cis*. Since the configuration at the Δ^6 bond does not appear to be critical, the participation of the *trans–cis* isomer (VIIc) is postulated. The optimal arrangement for formation of the cyclopentane ring is apparently the



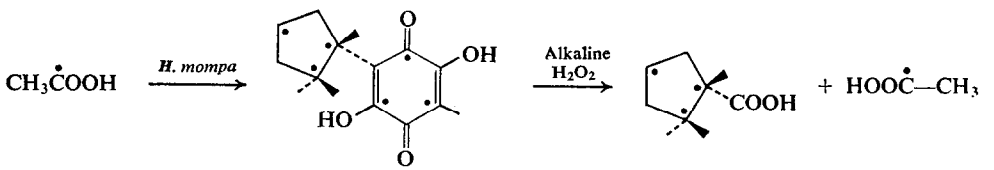
(VIIc) (XI) (XII) (XIII)

* For tricothecin biosynthesis, where bisabolene is again a possible precursor, the results of tracer experiments have established (IIIb) rather than (IIIa) as the labelling pattern.[9,10]

[9] E. R. H. JONES and G. LOWE, *J. Chem. Soc.* 3959 (1960).
[10] W. O. GODTFREDSEN and S. VANGEDAL, *Acta Chem. Scand.* **19**, 1088 (1965).
[11] W. G. DAUBEN and P. OBERHÄNSLI, *J. Org. Chem.* **31**, 315 (1966).

boat-type folding indicated in (VIIc). (Compare the biosynthesis of tricothecin from bisabolene.[10]) The folding for the six-membered ring is roughly that of a half-chair conformation. Discharge of the cation (XI), likely by use of NADH or NADPH, would lead directly to the monounsaturated intermediate (XII). Oxidation to the (*S*)-cuprenene (III), or an isomer thereof with correct stereochemistry, would be followed by oxygenation. Helicobasidin obtained by this route from mevalonate-2-^{14}C would have the labelling pattern of (Ib), or if an intermediate allowing randomization were involved (Ic).

The degradation results with helicobasidin derived from mevalonate-2-^{14}C are consistent with either of the labelling patterns (Ia), (Ib), or (Ic). To distinguish between these possibilities, degradations giving radioactivity in the positions numbered 1 and 3 of (Ia) and (Ib) would be necessary, but are not presently available. The results of the experiment with acetate-1-^{14}C are also consistent with any of the hypotheses discussed here. In each case, the labelling pattern (If) would be obtained via *trans–cis*–farnesyl pyrophosphate. On degradation, the camphonanic acid (IIa) should have contained 50 per cent of the helicobasidin activity, and the acetic acid 17 per cent. The observed values were 48 and 15 per cent respectively.



(If) (IIa)

At present, therefore, a decision between the three alternatives must be based primarily on inherent probability. The direct concerted cyclization possesses an over-all simplicity. The bisabolene route requires a large number of transformations. Furthermore, the naturally occurring cuparene and also the cuprenene isomers so far isolated* are dextrorotatory and have (*R*) configuration at the chiral center.[11–13] On the other hand, this same center in helicobasidin (I) is of opposite chirality.[3] Hence those members of the cuprenene–cuparene series so far known as natural products are most unlikely to be helicobasidin precursors. It is, of course, possible for bisabolene to cyclize to a precursor with the correct stereochemistry. If this is so, the hypothetical pathway must be clearly specified as follows: bisabolene→(−)-cuprenene→(−)-cuparene→(−)-helicobasidin.

The route *via* the bicyclic cation seems unnecessarily complex, requiring a postulated contraction of a seven- to a six-membered ring (VIII)→(IX) and, as well, the contraction of all six-membered ring to a five-membered ring (IX)→(X).

Our results agree with those of Natori *et al.*,[14] published while this work was in progress. With mevalonate-2-^{14}C, a 0·11 per cent incorporation was obtained by these authors, and the camphonanic acid contained 68 per cent of the total activity.† The acetic acid fragment was not isolated, however. Thus, an isoprenoid origin has been established for helicobasidin.

* From oils of *Chamaecyparis thyoides* and *Thujopsis dolabrata.*

† Average value from two degradations.

[12] C. ENZELL and H. ERDTMAN, *Tetrahedron* **4**, 361 (1958).

[13] T. NOZOE and H. TAKESHITA, *Tetrahedron Letters* **23**, 14 (1960).

[14] S. NATORI, Y. INOUYE and H. NISHIKAWA, *Chem. Pharm. Bull.* **15**, 380 (1967).

Major pathways for biosynthesis of other benzoquinones involve shikimic acid or acetate plus polymalonate condensations.[15]

EXPERIMENTAL

Growth Conditions for Helicobasidium mompa

Helicobasidium mompa Tanaka, ATCC 11046, obtained from the American Type Culture Collection, was maintained on slopes of the following composition: yeast extract (Difco), 1 per cent; glucose, 5 per cent; agar (Difco), 2 per cent. In all cases, transfers were made by cutting out small pieces of agar and a portion of the colony. Experimental cultures were grown on 500-ml portions of the following medium contained in 2·8-l. Fernbach flasks: sucrose, 5 per cent; malt extract (Difco), 3 per cent; peptone (Difco) 0·1 per cent. Incubation was at 26–28°. The organism grew slowly and was usually harvested after 80 days.

Isolation of Helicobasidin

The separated mycelial pads were washed with water, blotted free from superfluous water, dried in an oven, and pulverized. The average weight of dry mycelium was 1·01 g per 100 ml of culture fluid. The mycelial powder was extracted by standing with cold hexane for 24 hr; the flasks were shaken from time to time and two such extractions were employed. After filtration, the combined extracts were evaporated to dryness and the residue was sublimed under reduced pressure. The orange-red sublimate was dissolved in ether and the solution was extracted with 2 N NaOH. The aqueous extract was acidified and the solid separating out was crystallized several times from methanol. Helicobasidin was obtained as orange-red needles, m.p. 191–193°, with u.v. and i.r. spectra identical to those described in the literature.[6–8] The yield of helicobasidin averaged 3·2 mg per g of dry mycelium.

Tracer Experiments

In each case, six flasks were used containing a total of 3 l. of medium. The precursors were added in a small volume of sterile water; a sterile pipet was used and the radioactive solutions were placed into the medium below the pads. The mevalonic lactone preparation was first treated with an equivalent amount of alkali to convert it to the salt. Radioactivity measurements were carried out by liquid scintillation counting. In all cases, the scintillator fluid had the following composition: 2,5-diphenyloxazole, 16·0 g; *p-bis*[2-(5-phenyloxazolyl)] benzene, 0·06 g; toluene, 2400 ml; absolute ethanol, 1512 ml.

Degradation of Labeled Helicobasidin

Radioactive samples of helicobasidin were diluted with carrier material to a total weight of 100 mg and were then dissolved in 0·1 N KOH (20 ml). H_2O_2 (50 per cent, 12 ml) was added at room temperature; the solution was allowed to stand for several hours until the characteristic violet-red color of the solution had disappeared. On acidification with H_2SO_4, a colorless material separated out. After crystallization from ethanol–water, this material was further purified by TLC (twice) on silica gel using *n*-butanol saturated with 1·5 N NH_4OH. Yields of camphonanic acid were in the range 5–9 mg. The material had m.p. 186–189°, not depressed in admixture with authentic (±)-camphonanic acid (we are grateful to Professor H. L. Lochte for this material). The acetic acid, present in the mother liquors from the oxidation, was recovered by steam distillation and was converted to its *p*-bromophenacyl ester as described by Vogel.[16] The derivative purified by TLC in $CHCl_3$ and crystallization from ethanol, had m.p. 85°. The yield of derivative averaged 12 mg.

Acknowledgements—This work was supported, in part, by research grant GM 08477 of the United States Public Health Service. We are grateful to Dr. I. M. Campbell for discussions of this work.

[15] See, *inter alia*, J. GLOVER, in *Biochemistry of Quinones* (edited by R. A. MORTON), p. 207, Academic Press, New York (1965); R. BENTLEY and I. M. CAMPBELL, in *Comprehensive Biochemistry* (edited by M. FLORKIN and E. H. STOTZ), p. 415, Elsevier, Amsterdam (1968); R. BENTLEY, in *Metabolism and Function of Lipids* (edited by S. J. WAKIL), Vol. 1, Academic Press, New York, in press.

[16] A. I. VOGEL, *Practical Organic Chemistry*, third edition, p. 362, Longmans Green, London (1956).