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# THE STEREOCHEMISTRY OF HYDROGEN ELIMINATION FROM 4-C OF MEVALONATE IN THE BIOSYNTHESIS OF OPHIOBOLINS

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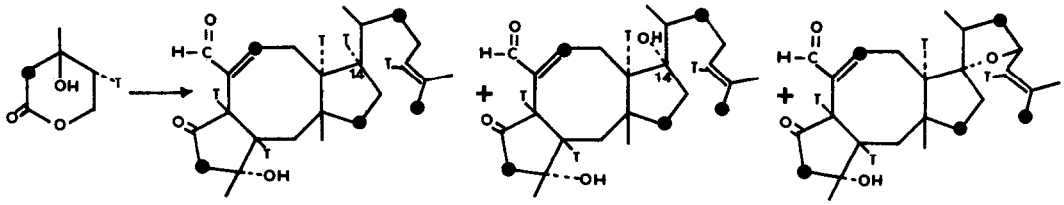
Ophiobolins (1) are substances constructed from five isoprene units linearly linked head to tail (2). We have shown previously that the carbonyl oxygens and those at 14-C of ophiobolin A 3 and B 2 came from the atmosphere, while oxygen from the medium appears in 3-C position (3). During biosynthesis of 2 and 3 the 1,5-shift of hydrogen occurs stereospecifically from 8α- to the 15-C position of the hypothetical intermediate 11 (3,4).



1 2 3

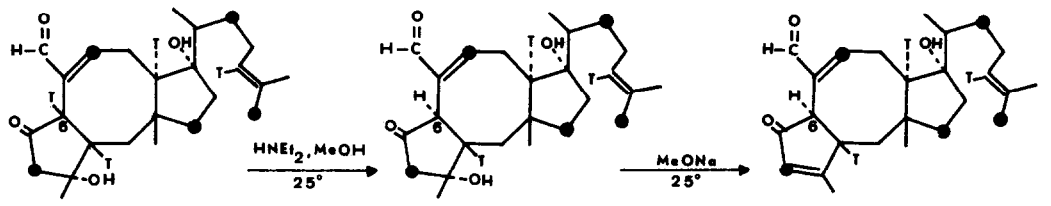
T = $^{3}H$ label ● = $^{14}C$ label

In order to examine some other stereochemical aspects in the biosynthesis of ophiobolins, either the 4R- or the 4S- isomer (±)-[2-$^{14}C$,4-$^{3}H$]- mevalonic acid lactone (9.8 μc $^{14}C$, $^{3}H/^{14}C$ = 6.62 and 10.2 μc $^{14}C$, $^{3}H/^{14}C$ = 7.57) was fed to one of two 300 ml shake suspensions of Cochliobolus miyabeanus (3). After two days the mycelium was harvested and found to contain 10 mg and 25 mg of ophiobolin C (5), respectively. $^{14}C$ incorporation in these two compounds were 0.22% and 0.54% (6) and $^{3}H/^{14}C$ ratios were 6.67 and 0.06, respectively. This corresponds to 101% retention of the 4R-hydrogen and only 0.8% retention of the 4S-

hydrogen.

Ophiobolin C is the biological precursor of ophiobolin B (7). In this step one of five tritium atoms i.e. that linked to 14-C is eliminated. In fact the isolated ophiobolin B 2 (195 mg, $^{14}C$ incorporation 3.71% (6)) showed $^{3}H/^{14}C$ = = 5.44. This corresponds to 103% of the calculated ratio for the presence in 2 of four tritium atoms out of five radioactive carbons.

After dilution with inactive material 2 was isomerized to 6-epiophiobolin B 4 (5) ($^{3}H/^{14}C$ = 4.06), which was transformed into anhydro-6-epiophiobolin B 5 (8) showing $^{3}H/^{14}C$ = 4.01. This corresponds to 102% of the calculated ratio for the presence of three tritium atoms out of five radioactive carbons, therefore the hydrogen at 6-C of 2 is labelled.




2 4 5

T = $^{3}H$ label ● = $^{14}C$ label

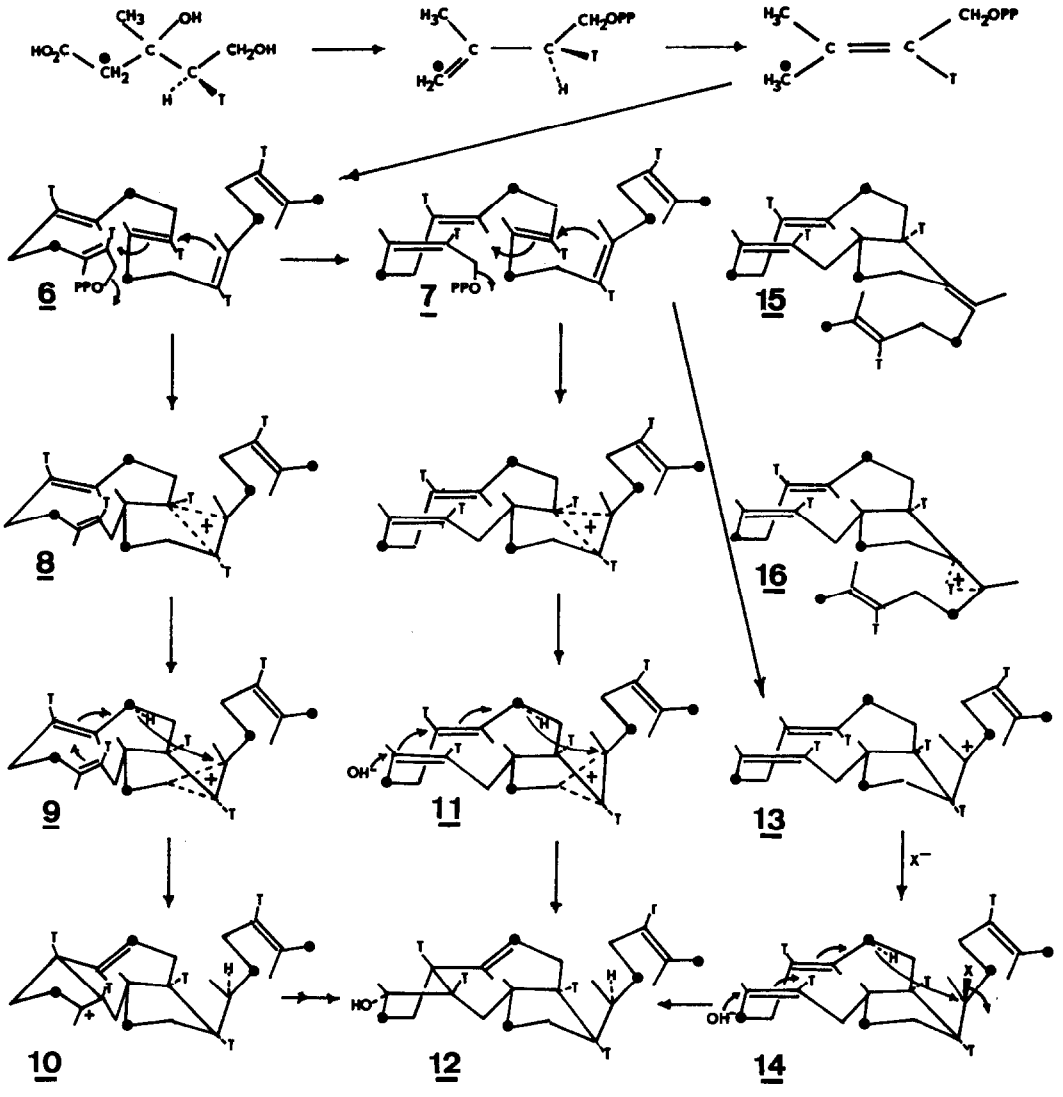
The $^{3}H$ and $^{14}C$ contents of the assayed products are reported in Table I.

Some conclusions can be drawn from our experiments:

a) owing to the S-hydrogen elimination and R-hydrogen retention of 4-C of mevalonate in the biosynthesis of ophiobolin C 1, the steric course of dimethylallyl pyrophosphate formation is 'normal', i.e. it occurs as in formation of the same compound in liver preparations (9). The condensation of isoprene units leads to the formation of trans-double bonds as in squalene in liver or in farnesol in latex of Hevea brasiliensis (9). As a consequence it seems likely that the biosynthesis occurs through the formation of all trans-geranylfarnesyl pyrophosphate 6 or its biological equivalent.

b) However the configuration of ophiobolins seems to require the cis-configuration of the acyclic intermediate 7 (3,4). This fact can be explained by the enzymic isomerization of 6 to 7. Alternatively 6 can yield, by solvolitic cycli-

zation through a concerted mechanism, the cation 8, which isomerizes to the cation 9. By saturation with the hydride ion arising from 8α-C, 9 yields the cation 10, which can transform to 12 in various ways. In both cases a non-stop cyclization takes place with formation of tricyclic intermediates 10 or 12.




We can also postulate an ionic intermediate as 13; this is possibly saturated by a nucleophilic center on enzyme or by solvent molecules to yield 14 which transforms to 12, for example by solvolysis and attack of hydrogen arising from 8α-C (10).

The other pathways proposed by Nozoe et al. (7) seem unlikely, because if 15 is formed, ophiobolin C 1 cannot contain the label found at 14-C. Moreover, on stereochemical grounds, it is very unlikely that hydrogen transfers from 8α-C to 15-C in 15 or 16.

c) As pointed out in our previous paper (3), if non-stop cyclization occurs, the $C_{8-9}$ double bond in tricyclic intermediates 10 or 12 seems to form in trans-configuration, because labelled hydrogen is linked to 6-C of isolated ophiobolins. We conclude that the very strained trans-cyclooctene isomerizes to the more stable cis-counterpart during the following biosynthetic pathway.

TABLE I

| SPECIMEN | RADIOACTIVITY (decs./min) | | |
|---|---|---|---|
| 4R-[2-$^{14}$C,4-$^{3}$H]-MVA standard | $^{3}$H | $^{14}$C | $^{3}$H/$^{14}$C |
| 0.2698 μg | 129,200 | 19,480 | 6.63 |
| 0.4047 μg | 195,200 | 29,490 | 6.62 |
| 0.5396 μg | 256,900 | 38,870 | 6.61 |
| Products from '4R'-mevalonic acid | | | |
| Ophiobolin C 1 (1.000 mg) | 14,780 | 2,214 | 6.67 |
| Ophiobolin B 2 (1.000 mg) | 10,660 | 1,961 | 5.44 |
| Ophiobolin A 3 (1.000 mg) | 11,490 | 2,097 | 5.48 |
| Ophiobolin B 2 (1.000 mg), dil. | 4,048 | 749 | 5.40 |
| 6-epiophiobolin B 4 (1.000 mg) | 3,017 | 743 | 4.06 |
| Anhydro-6-epiophiobolin B 5 (1.000 mg) | 3,055 | 762 | 4.01 |
| 4S-[2-$^{14}$C,4-$^{3}$H]-MVA standard | | | |
| 0.2564 μg | 139,300 | 18,390 | 7.57 |
| 0.3846 μg | 202,700 | 26,730 | 7.58 |
| 0.5128 μg | 269,060 | 35,580 | 7.56 |
| Products from '4S'-mevalonic acid | | | |
| Ophiobolin C (1.000 mg) | 142 | 2,269 | 0.06 |
| Ophiobolin B (1.000 mg) | 87 | 2,112 | 0.04 |

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