Tetrahedron Letters No.19, pp. 2347-2349, 1968. Pergamon Press. Printed in Great Britain.

# BIOSYNTHESIS OF OPHIOBOLINS FROM THE DOUBLY LABELED MEVALONATE (1)

Shigeo Nozoe, Masuo Morisaki, Shigenobu Okuda and Kyosuke Tsuda*

Institute of Applied Microbiology, University of Tokyo

Bunkyo-ku, Tokyo, Japan

(Received in Japan 17 January 1968; accepted for publication 13 February 1968)

We previously reported the results of $^{18}O$ incorporation experiments which indicated the biogenetical origin of the oxygen atoms in ophiobolin (2), and made some speculation concerning the cyclization processes of the hypothetical precursor, geranylfarnesyl pyrophosphate, to ophiobolin. However, saturation mechanism of the cationic center at C-15 produced by solvolytic cyclization of the prenylpyrophosphate has been obscure. We wish to report further information regarding this cyclization, obtained by tracer experiment using the doubly labeled substrate, 4R, 4-$^{3}H$-2-$^{14}C$-mevalonic acid lactone. In the biosynthesis of multiprenyl alcohol, it has been shown (3) that 4S proton of mevalonic acid is eliminated stereospecifically, while 4R proton retains in trans-multiprenyl chain. The fate of these retained hydrogens, when $C_{25}$-pyrophosphate is converted into ophiobolin, is of interest.

The washed micellium of Cochliobolus heterostrophus precultured in a potato broth medium at 26° for 70 hours was suspended in citrate-phosphate buffer pH 5.6 containing 5% of sucrose. An aqueous solution of 4R, 4-$^{3}H$-2-$^{14}C$-mevalonic acid lactone (4) ($^{3}H$, $3.6\times10^{7}$ cpm., $^{14}C$, $2.6\times10^{7}$ cpm.) was added to the suspension and incubation was continued for a further 45 hours at 26.5°.

After the usual processing, the crude materials isolated were separated by silica-gel chromatography. Ophiobolin-A (I), -B (II), -C (III) and ergosterol were purified rigorously to constant activity by repeated crystallization with carrier substances. Ophiobolin-A (I) and -C (III) were transformed chemically into pyridazine derivative IV and V respectively by treatment with hydrazine

---

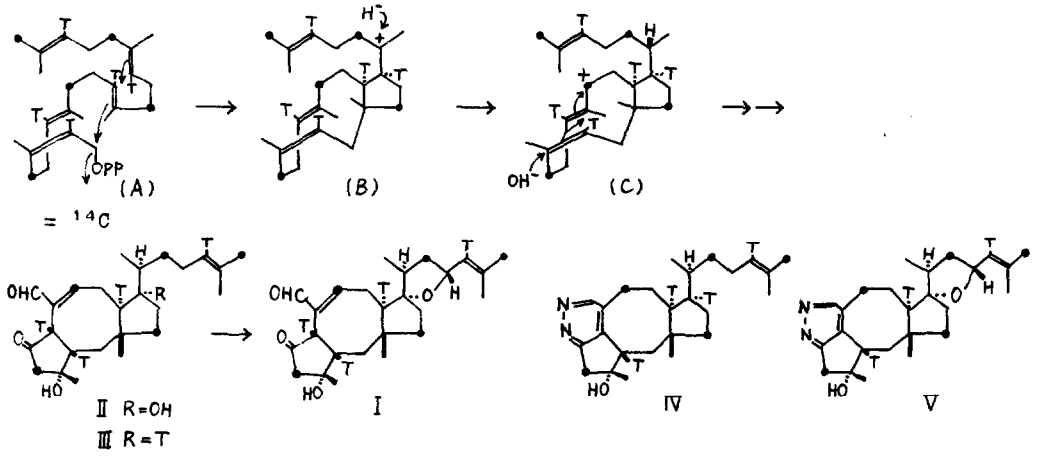
* Present address: Kyoritsu Pharmaceutical College, 6 Shiba-Koen, Minato-ku, Tokyo

monohydrochloride (2). Table I summarizes the measurement of radioactivity which were obtained using a Packard Tri-Carb liquid scintillation counter.

Table I

| | $^{14}C$ cpm | $^{3}H$ cpm | $^{3}H/^{14}C$ | corrected $^{3}H/^{14}C$ |
|---|---|---|---|---|
| MVA benzhydrylamide | 1060 | 1500 | 1.42 | ---- |
| Ergosterol | 710 | 602 | 0.848 | 2.99 : 5 |
| Ophiobolin-A | 4160 | 4770 | 1.15 | 4.03 : 5 |
| Ophiobolin-B | 830 | 940 | 1.13 | 3.98 : 5 |
| Ophiobolin-C | 862 | 1240 | 1.44 | 5.06 : 5 |
| Pyridazine deriv. of A | 642 | 534 | 0.832 | 2.93 : 5 |
| Pyridazine deriv. of C | 530 | 594 | 1.12 | 3.95 : 5 |

As shown in Table I, all tritium atoms have been retained in ophiobolin-C, while one of the five, that on C-14 position, has been lost in ophiobolin-A and -B. The ratio of $^{3}H$ and $^{14}C$ in the pyridazine derivatives IV and V also comfirmed these facts. Therefore, tritium atom on C-14 position of ophiobolin C, should have retained its original attachment. These findings exclude the possibility of the saturation mechanism by deprotonation-reduction pathway via $\Delta^{14(15)}$-compound (2) or 1, 2-H-shift from C-14 to C-15 position followed by $OH^-$ or $H^-$ attack at C-14 position. It is reasonable to assume that the cationic center is reduced by hydride attack at C-15 position.



Recently L. Canonica et al. (6) reported the evidences of the stereospecific 1, 5-hydride shift from 8α to 15 position during ophiobolin biosynthesis. Present results are not inconsistent with their findings, thus, hydride ion in (B) must be produced intramolecularly and the resulted cation in (C) initiates second cyclization as proposed by L. Canonica (6).

The $^{3}H/^{14}C$ ratio in ophiobolins and pyridazin derivatives also indicated that stereochemistry of the A/B ring junction was original one since no tritium loss was observed at C-6 position in ophiobolin.

It was also comfirmed that the $^{3}H/^{14}C$ ratio of the ophiobolin A, B, and C, biosynthesized from 2S, 2-$^{3}$H-2-$^{14}$C-mevalonic acid lactone has been found all 5:5. Investigations are under progress using the doubly labeled substrate, 2R, 2$^{3}$H-2-$^{14}$C-mevalonic acid lactone.

## References


1. The authors thank Dr. L. Canonica for making their paper of double tracer work available to us prior to publication.
2. Shigeo Nozoe, Masuo Morisaki, Kyosuke Tsuda and Shigenobu Okuda: Tetrahedron Letters, No. 35, 3365 (1967).
3. G. Popjak and J. W. Cornforth: Biochem. J., 101, 553 (1966).
4. J. W. Cornforth, R. H. Cornforth, C. Donninger and G. Popjak: Proc. Roy. Soc., B 163, 492 (1966).
5. Cationic center was tentatively mentioned as classical form at $C_{15}$, although various participations can be considered.

6a. L. Canonica, A. Fiecchi, M. Galli Kienle, B. M. Ranzi, A. Scala, T. Salvatori and E. Pella: Tetrahedron Letters, 3371 (1967).

6b. L. Canonica, A. Fiecchi, M. Galli Kienle, B. M. Ranzi and A. Scala: ibid, 4657 (1967).