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BIOGENESIS OF OPHIOBOLINS. THE ORIGIN OF THE OXYGEN ATOMS IN THE OPHIOBOLINS (1)

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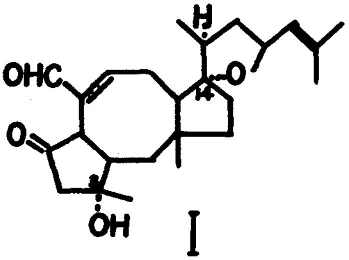
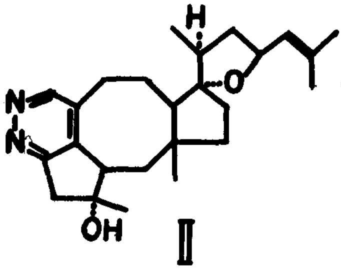
We previously reported the isolation and the structural elucidation of ophiobolin-A (2), -B (3), -C (4), and -D (4,5), metabolites of plant pathogenic fungi. These compounds are believed to be the first examples of C_{25}-terpenoids in nature. Ophiobolins are considered to be produced from geranylfarnesyl pyrophosphate or its biochemical equivalents, which in turn, might be constructed from five isoprene units. Recently, L. Canonica et. al. reported (6) degradation studies on radioactive ophiobolin biosynthesized from 2-^{14}C-mevalonate to prove this hypothesis.

It has been suggested (2) that the five-eight-five-membered ring system of ophiobolin might be constructed by a successive two-step cyclization, in which the first step was assumed to be a solvolytic cyclization leading to the five-eleven-membered skeleton and the second one a cation-induced anti-parallel addition leading to the carbon skeleton of ophiobolin. In relation to this cyclization scheme, the problems to be solved were (a) does the oxygen function at the C_{14} position in ophiobolin-A and -B participate in the first cyclization step, and (b) does the hydroxyl group at the C_3 position initiate the second cyclization step? The present studies provide the evidences as to the origin of these two oxygen atoms.

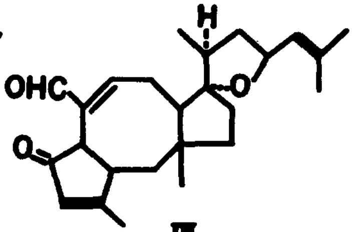
The micellium of Cochliobolus heterostrophus, precultured for 25 hours under the usual conditions, was transferred with the medium into a flask, which was then evacuated and filled with a mixture of ^{18}O enriched oxygen (10% ^{18}O) and nitrogen. The flask was then shaken at 26° for 48 hours. After the usual processing, the crude material isolated was purified by silicagel chromatography to obtain the pure sample of ophiobolin A (I). Treatment of I thus obtained with hydrazine hydrochloride in methanol in the presence of sodium acetate afforded the pyridazine compound II, m.p. 178-180°, $C_{25}H_{36}O_2N_2$, M^+=396, λmax 225, 256, 300 mμ (ε, 5,000, 2,500, 540): n.m.r. peaks at δ8.70 (singlet, 1H). The mass spectrum of compound II was specifically analyzed (7) in the region of the molecular ion peak (base peak) and its isotope peak. Comparison of the increased height of the M+2 ion peak with that of the M ion peak indicated the incorporation of ^{18}O into the one of two oxygen atoms of pyridazine derivative II. On the other hand, the ophiobolin A (I), which was biosynthesized under the atmosphere of ^{18}O enriched oxygen was converted to anhydroophiobolin A (III), m.p. 136-137°, $C_{25}H_{34}O_3$, M^+=382, λmax 227.5 mμ (ε, 23,600), ν max 1695, 1645, 1620 cm^{-1}: n.m.r. peaks at δ2.04 (3H, singlet, C_3-Me), 3.20 (1H, doublet, C_6-H), 5.90 (1H, broad singlet, C_4-H), with hydrochloric acid in tetrahydrofuran. The compound III thus obtained was dissolved in methanol-acetone-H_2O mixture containing hydrochloric acid and the solution was allowed to stand for two days at room temperature to exchange the labile oxygen atoms. After purification of the product by chromatography, the sample was also analyzed mass-spectrometrically. It was found that one stable oxygen atom in III contained ^{18}O. These results confirmed that the oxygen atom at the C_{14} position was derived from atmospheric oxygen, and that the hydroxyl group at the C_3 position did not originate from molecular oxygen.



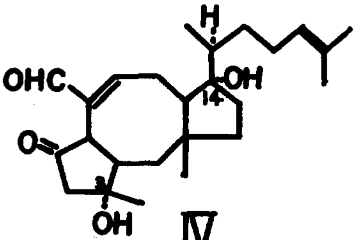
I



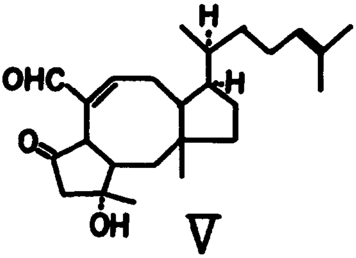
II



III

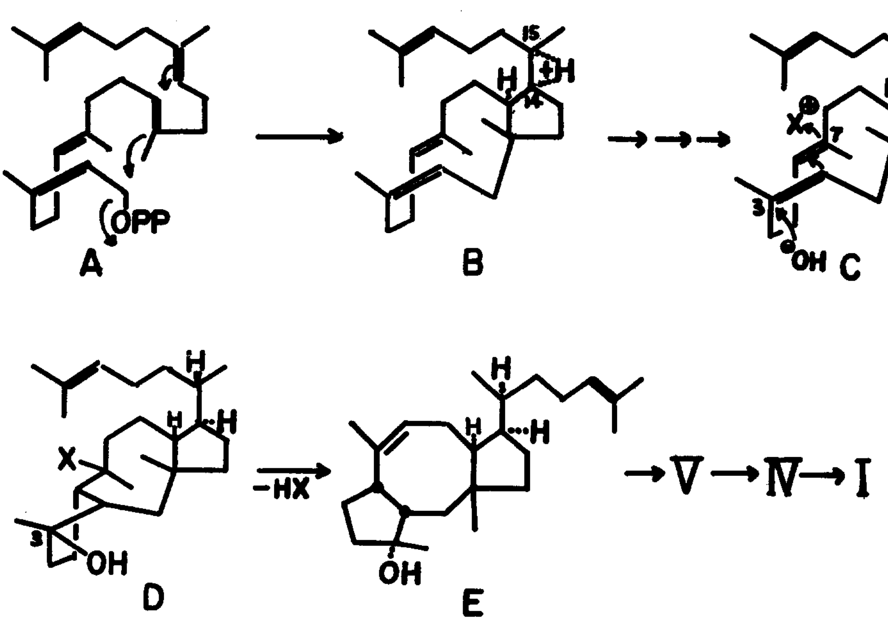


IV



V

These findings are consistent with the results of the experiments on incorporation of radioactive materials. Tritiated ophiobolin C (V) (specific activity 3×10^7 dpm/mg.), prepared by the Wilzbach method was added (1.98×10^7 dpm) to the cultured medium of Cochliobolus heterostrophus after 30 hours growth and incubation was continued for a further 46 hours. Ophiobolin A was isolated from the cultured broth and purified by several recrystallizations to a constant specific activity (2.5×10^3 dpm/mg.). To the filtrate, cold ophiobolin B (IV) was added as carrier and the isolated IV was purified by several recrystallizations to constant specific activity (1.5×10^2 dpm/mg.). These results revealed that ophiobolin A (I) was produced from ophiobolin C (V), probably via ophiobolin B (IV) (5) by simple hydroxylation at the 14α position and the subsequent formation of an ether linkage. In other words, the hydroxyl group at the C_{14} position of IV does not participate in the cyclization to neutralize the cationic center in (B). In connection with the above results, the following possibility for the cyclization of the precursor (A) is conceivable.



In this scheme, the cationic center at the C_{14}-C_{15} position genarated in the first-step solvolytic cyclization is directly or indirectly (e.g.via unsaturated compound produced by deprotonation) reduced to a saturated C_{14}-C_{15} linkage to give an intermediate such as (C). Since the ^{18}O-incorporation study indicated that the hydroxyl group at the C_3 position does not originate from atmospheric oxygen, the second-step cyclization must be initiated at the C_7 position as shown in (C), and the alternative possibility (2)——$OH^{\oplus}$ intiated cyclization at C_3 position——can be excluded.

Further experiments are now under investigation in our laboratory to clarify the hypothesis in more detail.

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