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THE BIOSYNTHESIS OF OPHIOBOLINS

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Ophiobolins A and B (1) are substances constructed from five isoprene units linearly linked head to tail (2). In their biogenetic hypothesis Nozoe et al. proposed that the introduction of a 3-hydroxyl substituent is accounted for by attack at 3-C by an agent which transfers OH^+ (3).

To prove this hypothesis, Cochliobolus miyabeanus was cultured in a synthetic medium. After two days growth, the mycelium was centrifuged and then shaken for 3 1/2 days in a medium lacking in nitrogen compounds and in air containing ^{18}O (4.55%) enriched oxygen.

^{18}O enriched ophiobolin B 1 was transformed in carbon dioxide by a modified Unterzaucher method (4) and the ^{18}O enrichment determined by mass-spectrometry. The number of ^{18}O enriched atoms per molecule was calculated with the formula $N = (x - 0.00204)T / 0.0435$ where $(x - 0.00204)$ and 0.0435 are ^{18}O atom fraction excess in the analyzed compound and in the enriched air respectively, T is the total number of oxygen atoms in the analyzed compound and x is calculated according to Miller (5). By this method we found N = 2.5 for 1; this fractional value is probably caused by a partial exchange with the medium of the carbonyl oxygens during biosynthesis and isolation of 1.

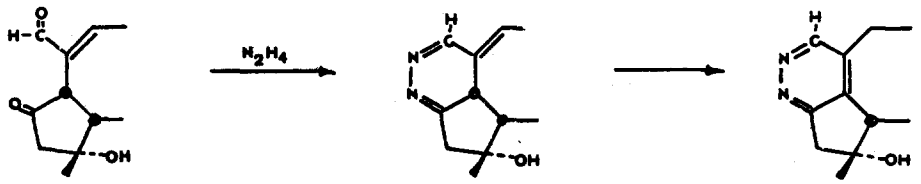
Ophiobolin B 1 was dehydrated in alkaline medium containing ^{18}O enriched water; we found N = 1.5 for the anhydro-ophiobolin B 2. A partial exchange (75%) of the two carbonyl oxygens was thus proved.

In the same conditions ^{18}O enriched ophiobolin B 1 was dehydrated in alkaline medium containing tap water. We found N = 1.2 for anhydro-ophiobolin B 2. This result shows that in 2 one ^{18}O enriched oxygen is linked to 14-C and 0.2

enriched atoms are localized on carbonyl oxygens, owing to partial exchange with the medium.

^{18}O enriched ophiobolin A 3 contained ^{18}O enriched oxygen linked to 14,17-C, 5-C and 21-C, as shown by the high intensity of the peaks at m/e 167 = $C_{11}H_{17}{}^{18}O$ (6) and at m/e 178 = $C_{11}H_{12}{}^{16}O{}^{18}O$ (7) present in its mass spectrum.

With excess hydrazine ophiobolin A 3 yields the methine-pyridazine 4; m.p. 188-190°; $C_{25}H_{36}O_2N_2 \cdot 1.5H_2O$; M^+ 396; UV spectrum λ_{max} 278 mμ, ε 8450; IR spectrum ν_{max} 3400, 3300, 1660, 1640 cm^{-1}; NMR spectrum in d_5-pyridine shows signals at δ 7.5 ppm (singlet, 1 H, 21-CH) and 6.2 ppm (multiplet, 1 H, 7-CH). In common solvents at room temperature 4 yields 5; m.p. 168°; $C_{25}H_{36}O_2N_2$; M^+ 396; UV spectrum λ_{max} 228 mμ, ε 4700, λ_{max} 253 mμ, ε 2100, λ_{max} 297 mμ, ε 470; IR spectrum ν_{max} 3380, 3320, 1590 cm^{-1}; NMR spectrum in d_5-pyridine shows the signal of 21-CH as a singlet at δ 9.1 ppm.



3 4 5

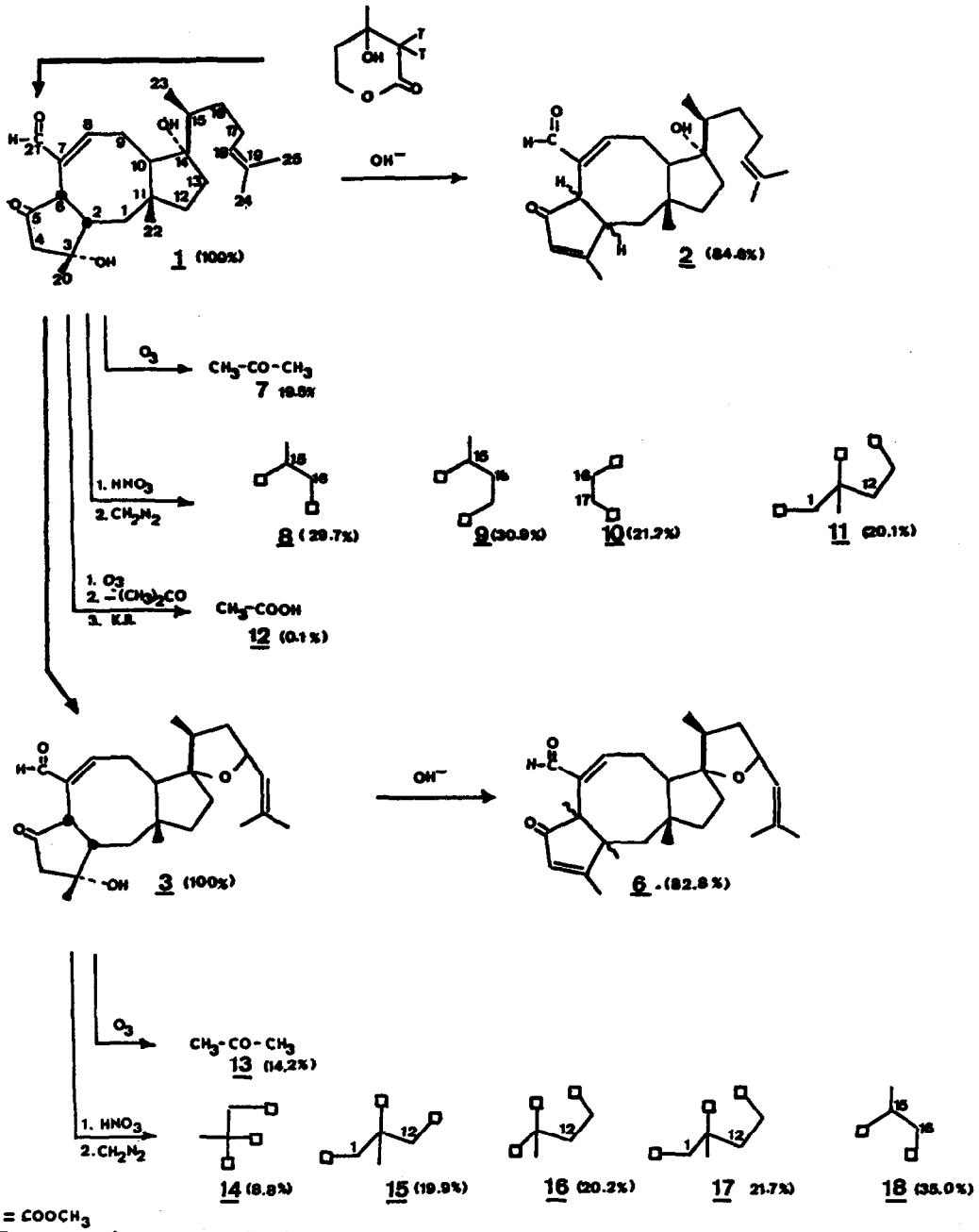
In the same way ^{18}O enriched ophiobolin A 3 yielded ^{18}O enriched pyridazine 5 containing 1.1 ^{18}O enriched oxygen atoms

These results show that in the biosynthesis of ophiobolins A and B molecular oxygen is directly incorporated into the 14-C, while on the contrary oxygen from the medium appears in 3-C position. Therefore the proposed hypothesis of Nozoe et al. (3) is incorrect, assuming that during biosynthesis the 3-OH does not exchange with the medium.

The biosynthetic pathway of ophiobolins was further studied in the following manner: Cochliobolus miyabeanus was cultured as described above, DL 2-^3H mevalonic acid lactone (0.2 mC) being added after two days growth. After 3 1/2 days ophiobolin A 3 (46 mg, $3.37 \cdot 10^{-2}$ μC/mg, incorporation 2.36% (8)) and ophiobolin B 1 (268 mg, $5.38 \cdot 10^{-2}$ μC/mg, incorporation 14.42% (8)) were obtained.

After dilution with inactive material, ophiobolin A 3 and B 1 were degraded through the pathways reported in table 1. Comparison of the activities of 1 and 3 and of anhydroderivatives 2 and 6 indicated that tritium was linked to 4-C. This result shows that during biosynthesis 3-OH does not exchange with the medium through dehydration. The high activities of 2 and 6 are explained by consi-

TABLE 1.*



□ = $COOCH_3$

*In parentheses are indicated the molar radioactivities expressed as percentages of molar radioactivity of 1 and 3 respectively.

dering that during dehydration only 80% of the hydrogens on 4-C exchange with the medium (9).

Comparing the activities of 16 and 17 we notice that 1-CH_2 is not labelled. 13-CH_2 too does not contain tritium, in fact 15 and 16 have the same label. Therefore tritium is linked 12-C as it also appears from the weak radioactivity of 14 owing to its methylene group comes either from 1-CH_2 or 12-CH_2. The activity of acetone phenylthiosemicarbazone 7 shows that tritium is linked to 24- or 25-C.

In this way we are able to localize tritium on the carbon atoms 4-, 12- and 24- or 25-. The residual activity can be on the hydrogens of the carbons 2-, 8-, 9-, 10-, 15-, 16-, 17-, 18- and 21-, being excluded the hydrogens on the carbons 20-, 22- and 23- because the acetic acid 12 obtained by Kuhn-Roth oxidation of ozonized ophiobolin B 1 was not labelled.

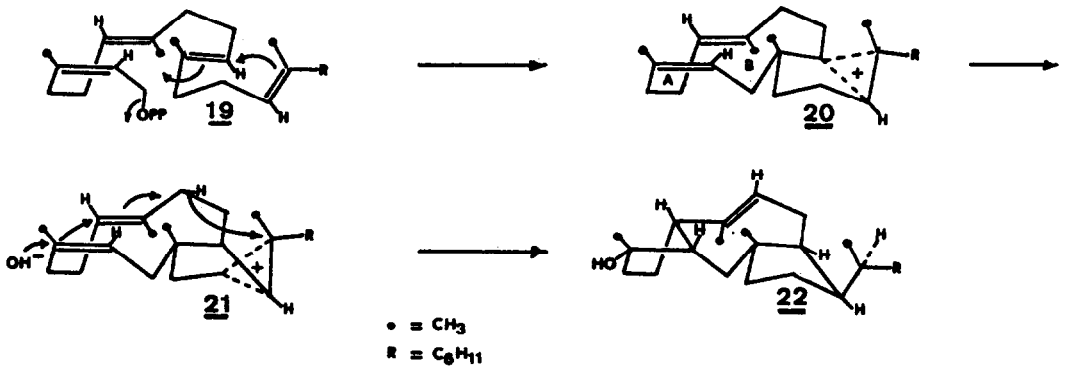
We have found that S(-)methyl methylsuccinate 8 and S(+)methyl 2-methylglutarate 9 contain 1 1/2 times the radioactivity of 7, 10 and 11 whereas on biogenetic grounds (2) we should have found the same value, corresponding to two tritium atoms. Therefore 8 and 9 contain three labelled hydrogen atoms. By comparing radioactivity of 8, 9 and 10 (10 is derived almost completely from 15-, 16-, 17- and 18- carbon atoms) we can localize two tritium atoms on 16-C. We can also localize the third labelled atom of 8 and 9 on 15-C; in fact acetic acid 12 is not radioactive.

Owing to the unexpected label on the hydrogen linked to 15-C we have localized nine tritium atoms in 1. Furthermore the found values of 2 (allowing for the partial exchange with the medium), 7, 8, 9, 10 and 11 agree with the presence in 1 of ten rather than nine tritium atoms. On biogenetic grounds we believe that the tenth tritium atom is linked to 8-C (2). Therefore we conclude that during biosynthesis tritium is not lost and probably one hydrogen transfers from 8-C to 15-C.

The same conclusions can be drawn from the found activities of 6 (allowing for the partial exchange with the medium), 17 and 18, provided that during the biosynthesis of 3 from 1, 24-CH_3 (10) exchanges a hydrogen atom. In fact acetone phenylthiosemicarbazone 13 from ophiobolin A 3 contains only 14.2% of the total activity. This value agrees with the presence in this fragment of 1.33 labelled hydrogens out of 9.33.

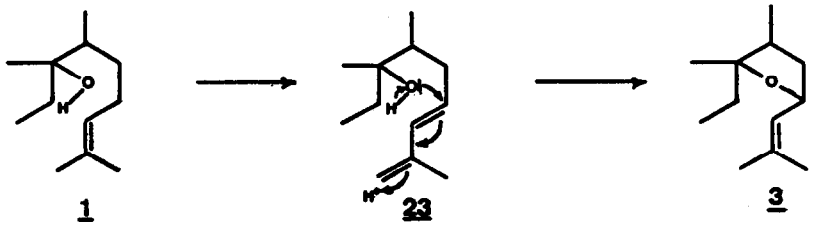
From our experiments it is now possible to rationalize the biosynthetic pathway to ophiobolins: geranylfarnesylpyrophosphate or its biological equivalent 19 yields by solvolitic cyclization, through a concerted mechanism, the cation 20, which isomerizes to the cation 21 (11). By saturation with the hydride

ion arising from 8-C, 21 yields the hypothetical intermediate 22, from which all the ophiobolins can be derived. The absolute configuration of 22 is the same reported for ophiobolane (1).



The C_{8-9} double bond has the trans-configuration in this intermediate, whereas in the ophiobolins the C_{8-9} double bond is in cis-configuration. Moreover the very strained trans-cycloöctene can easily isomerize to the more stable cis--counterpart during the following biogenetic pathway. Alternatively it is possible to hypotize a cyclic intermediate with a cis-configuration for C_{8-9} double bond and a trans-fusion between A and B rings with 6-αCH. This asymmetric center should isomerize to 6-β configuration during biosynthesis; on the other hand, this fact does not seem probable because the α-configuration is the most stable in the ophiobolins (9).

As described above, the exchange of a hydrogen of 24-CH_3 occurs during biosynthesis of ophiobolin A 3 from ophiobolin B 1. We were unable to incorporate one of the possible intermediates, i.e. 17,18-epoxyophiobolin B (12).



At present a hypothetical intermediate like 23 seems to account for our experimental results.

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