Enzymic Formation of a Tricyclic Sesterterpene Alcohol from Mevalonic Acid and all-*trans*-Geranylfarnesyl Pyrophosphate

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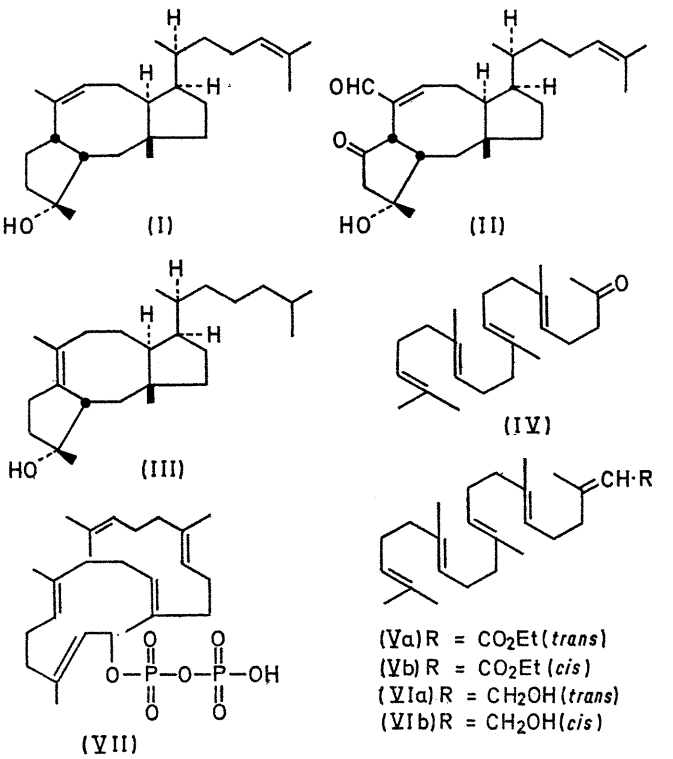
Summary Mevalonic acid lactone and the chemically synthesized all-*trans*-geranylfarnesyl pyrophosphate are converted into a tricyclic sesterterpene alcohol, ophiobolin F, by incubation with 100,000 × *g* supernatant fraction from cell-free system of *Chochliobolus heterostrophus*.

A PHYTOTOXIC substance, ophiobolin A, isolated from the plant pathogenic fungi, was the first known sesterterpene.[1] Later, several related compounds were found to occur in fungal metabolites[2] or in insect secretions.[3] Biosynthetic studies of the ophiobolins using the intact cell system were reported and experimental evidence for the genesis of these compounds was presented.[4,5] We have found that a crude enzyme system obtained from the cell-free homogenate of the *C. heterostrophus* catalysed the formation of the tricyclic sesterterpene alcohol (I) from MVA and all-*trans*-geranylfarnesyl pyrophosphate.

Mycelia of *C. heterostrophus* were ground in a mortar with sand and 0·1M-phosphate buffer (pH 7·35) containing $MgCl_2$, and the homogenate was successively centrifuged, first at 1000 × *g*, for 10 min., then at 100,000 × *g* for 1 hr. The 100,000 × *g* supernatant was incubated with [2-^{14}C]-MVA in the presence of ATP, for 3 hr. at 37°. After the addition of KOH, the incubation mixtures were extracted with ether, and the extracts were chromatographed on silica-gel plates. 56% of the radioactivity of the added substrate appeared in a band having the same R_F value as ophiobolin F (I)[6] which had been isolated from the same fungus as a very minute component, and its structure was tentatively assigned as (I). The conclusive identification of the enzymic reaction product and ophiobolin F is based on autoradiography on t.l.c., gas–liquid radiochromatography, and g.l.c. mass spectral analysis.

The structure and stereochemistry of ophiobolin F (I) were confirmed by chemical correlation with ophiobolin C (II), whose structure and absolute configuration were already established.[7] Reduction of ophiobolin C (II) with lithium



aluminium hydride afforded a triol, m.p. 102—104°, which was hydrogenated with palladium–charcoal in ethanol to give the mono-alcohol derivative (III), $C_{25}H_{44}O$, M^+ 360, intense peaks at *m/e* 345, 342, 327, 257, 247, 229, and 207.

The n.m.r. spectrum of (III) exhibited signals at δ 0·79, 0·82, 0·89 (9H, three secondary methyls), 0·97 (s, C-11 Me), 1·13 (s, C-3 Me) and at 1·52 (s, C-7 Me). The identical mono-alcohol (III) was also derived from ophiobolin F by catalytic hydrogenation.

All-*trans*-geranylfarnesyl pyrophosphate was synthesized by a known procedure, as follows. Condensation of the all-*trans*-geranylgeranylacetone (IV)[8] (prepared from geranyl-linolool with triethyl phosphonoacetate in tetrahydrofuran in the presence of sodium hydride) afforded ethyl geranylfarnesoate (V) as a *cis,trans*-mixture in a ratio of *ca.* 1 : 4. Chromatographic separation of the mixture yielded all-*trans*-ethyl geranylfarnesoate (Va), [(M^+ 400), n.m.r. signals of methyl groups appeared at δ 1·58 (12H), 1·67 (3H), 2·12 (3H)] and a terminal *cis*-isomer (Vb), [(M^+ 400), n.m.r. signals at δ 1·58 (12H), 1·66 (3H), 1·88 (3H)] both of which were reduced by lithium aluminium hydride to give all-*trans*- and a terminal *cis*-geranylfarnesol (VIa, VIb), respectively. All-*trans*-isomer (VIa) showed methyl proton signals at δ 1·58 and 1·66 which differ from the reported value for natural geranylfarnesol recently isolated from insect wax.[9] The natural compound was assumed to be a terminal *cis*-isomer from a comparison of the its n.m.r. spectrum with that of the synthetic compound which exhibited signals at δ 1·58, 1·66, and 1·72. Tritium-labelled geranylfarnesol obtained by lithium aluminium tritiide reduction of (Va) was converted into the pyrophosphate ester (VII) by Cramer's procedure as modified by Kandutsch *et al.*[10] The synthetic [1,1-$^3H_2$]-all-*trans*-geranylfarnesyl pyrophosphate (VI) was incubated with 100,000 × *g* supernatant fraction under the same conditions described above to give ophiobolin F (I) (*ca.* 20%). Negligible incorporation of radioactivity into (I) was observed when [1,1-$^3H_2$]-*cis*-geranylfarnesyl pyrophosphate and [1,1-$^3H_2$]-all-*trans*-geranylfarnesol were used as substrates. The data presented provide the first experimental evidence for the intermediary role of geranylfarnesyl pyrophosphate in sesterterpene biosynthesis. Investigations on co-factor requirements, substrate specificity, and the separation of cyclizing enzyme activity from the others are in progress.

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