# Studies in Relation to Biosynthesis. Part XXXVIII.[1] A Preliminary Study of Fumagillin

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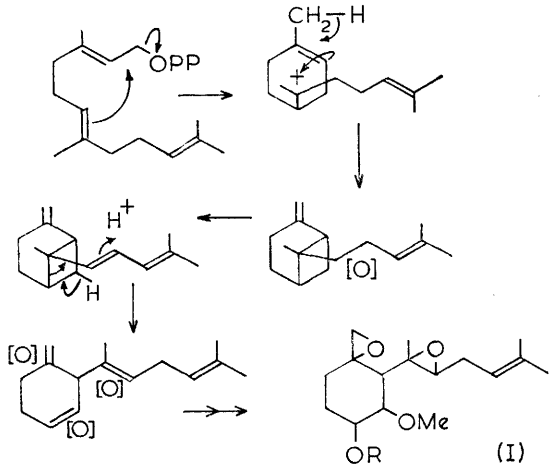
The nucleus of fumagillin (I; R = CO·[CH=CH]$_4$·CO$_2$H) is shown by tracer studies to arise by the terpene route. The side-chain is produced from ' acetate ' without intervention of a symmetrical intermediate.



FUMAGILLIN [I; R = CO·[CH=CH]$_4$·CO$_2$H) is an antibiotic from *Aspergillus fumigatus*.[2] By inspection of the formula it appears to arise by two routes from acetyl coenzyme-A: the terpene and the polyketide (or related fatty acid) route. However, the $C_{15}$-nucleus is not classically isoprenoid and could come in part from terpene precursors and in part from ' acetate '. A possible biosynthesis based on the more probable complete terpenoid origin is given in the Scheme.




SCHEME

Incorporations have therefore been examined of [$^{14}$C]acetic acid, known to be a precursor of both terpenes and polyketides, *e.g.*[3] and of [$^{14}$C]mevalonic lactone known to produce only the terpenoid portion in mixed terpene-polyketide molecules.[3]† The extent of incorporation was small, but sufficient to establish origins.

Fumagillin can be hydrolysed to (I; R = H) but owing to the low m.p. of this it is difficult to purify and to assay by our usual solid-counting technique. The radioactivity of (I; R = H) was therefore calculated by difference between the fumagillin and the sebacic acid produced by hydrolysis and hydrogenation.[2] The tracer results are expressed in relative molar activities,[4] preceded by the counts per 100 sec. for a 1 sq. cm. sample of infinite thickness (see Experimental Section). Derived from [1-$^{14}$C]acetic acid the antibiotic (c. sec.$^{-1}$ cm.$^{-2}$ 71·2, r.m.a. 3·21 × 10$^4$) gave sebacic acid [2] (c. sec.$^{-1}$ cm.$^{-2}$ 61·8, r.m.a.[4] 1·24 × 10$^4$) leading to r.m.a. 1·97 × 10$^4$ for (I; R = H). On the basis of six equally labelled units in (I; R = H) and five units in the acid, the extent of incorporation is different in the two portions of the molecule. This result agrees with similar differences between terpene and polyketide portions of the same molecule in other cases (see ref. 3). Decarboxylation of the sebacic acid by the Schmidt procedure gave carbon dioxide (barium carbonate c. sec.$^{-1}$ cm.$^{-2}$ 6·7, r.m.a. 0·13 × 10$^4$) consistent with five labels randomised over the 10 carbons because the symmetry renders the terminal carboxyls chemically equivalent although different in origin. To isolate only one of them, fumagillin was hydrogenated and submitted to Schmidt decarboxylation; the carbon dioxide as barium carbonate then had c. sec.$^{-1}$ cm.$^{-2}$ 11·75, r.m.a. 0·22 × 10$^4$, compared with the theoretical r.m.a. 0·24 × 10$^4$ for one labelled unit. A free dicarboxylic acid is therefore not involved in the biosynthetic esterification but possibly a monocoenzyme-A ester formed by ω-oxidation, possibly of decantetraenoic acid. In this hope, the latter was synthesised with [1-$^{14}$C]CO$_2$H. The product of incorporation showed, however, considerable label in both parts and presumably results from prior degradation into acetic acid. In support of the general conclusion [2-$^{14}$C]acetic acid was fed, and the resulting fumagillin (c. sec.$^{-1}$ cm.$^{-2}$ 22·6, r.m.a. 10·3 × 10$^3$) gave active decantetraendioic acid [2] (c. sec.$^{-1}$ cm.$^{-2}$ 11·0, r.m.a. 2·2 × 10$^3$), but on decarboxylation by the Schmidt procedure [5] it gave inactive carbon dioxide; the terminal carboxyl of fumagillin therefore does not arise at all from Me of acetate but entirely from the carboxyl.

Kuhn-Roth oxidation of the above fumagillin (r.m.a. 10·3 × 10$^3$) gave acetic acid, converted by the action of sodium azide and sulphuric acid into carbon dioxide (inactive) and methylamine (assayed as 2,4-dinitrophenyl-*N*-methylamine, c. sec.$^{-1}$ cm.$^{-2}$ 3·06, r.m.a. 0·6 × 10$^3$); the calculated value on the basis of a terpene origin of the nucleus is 0·67 × 10$^3$.

Higher incorporations were obtained with [2-$^{14}$C]-mevalonic acid. The fumagillin (c. sec.$^{-1}$ cm.$^{-2}$ 12·0, r.m.a. 5·49 × 10$^4$) gave (I; R = H) [2] together with inactive dicarboxylic acid. Ozonolysis of (I; R = H) [2] gave acetone, assayed as the 2,4-dinitrophenylhydrazone (c. sec.$^{-2}$ cm.$^{-1}$ 77·1, r.m.a. 1·8 × 10$^4$), carrying the

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calculated one-third of the activity for three isoprene units.

## EXPERIMENTAL

The [$^{14}$C]-precursors were fed to *Aspergillus fumigatus* through the generous assistance of Dr. J. C. Sylvester and Dr. R. U. Schock, who also provided fumagillin. The organism was grown in a standard medium and 100 μC of material added 24 hr. after inoculation, growth being continued for 7 days at 24°. The crude product was isolated [2] by pentyl acetate at pH 3·5 and the fumagillin isolated by carrier extraction with the pure dicyclohexylamine salt. Incorporation of [1-$^{14}$C]sodium acetate occurred to the extent of 0·21%, [2-$^{14}$C]acetate 0·2%, and [2-$^{14}$C]-mevalonic lactone 1·6%.

The degradations used were those in the previous literature [2] except for the Schmidt degradations which were carried out by the procedure of Phares.[5]

The tracer techniques were those used in previous Parts (see ref. 4) namely, solid samples of 0·3 sq. cm. were examined in an end-window counter, taking 10,000 counts with a statistical error of ±3%. R.m.a. of the order of $10^3$ or less were examined as carbon dioxide in an ion chamber of 100-ml. capacity and counted on a model 6000 Dynacon electrometer (Nuclear Chicago Corporation); by comparison with standards the results were brought to the same scale as those obtained with the end-window counter. The r.m.a. are defined [4] as the counts/100 sec. for 1 sq. cm. of a sample of infinite thickness multiplied by the molecular weight.

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