GEOSMIN AND METHYLISOBORNEOL BIOSYNTHESIS IN STREPTOMYCETES

Evidence for an isoprenoid pathway and its absence in non-differentiating isolates

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1. Introduction

Geosmin, 1, (*trans*-1,10-dimethyl-*trans*-9-decalol) and the 2-methyl derivative of isoborneol, 2, (1,2,7,7-tetramethyl-*exo*-bicyclo[2.2.1]heptan-2-ol) are volatile metabolites of various Streptomycetes, algae, and fungi [1]. Geosmin in particular has an intense 'earthy' or 'musty' odor. Both metabolites have caused odor and taste problems in water supplies [2] and foods but geosmin is important for the flavour of beets [3]. Despite the problems for water supplies, there is almost no biosynthetic information for these materials. However, aerial mycelium negative (amy$^-$) isolates of *Streptomyces alboniger, S. scabies*, and *S. violaceous-ruber* failed to produce an earthy odor; the uncharacterized material was assumed to be geosmin [4]. We have used radio–gas chromatography to investigate the biosynthesis of geosmin and 2-methylisoborneol and have obtained amy$^-$ isolates of *S. sulfureus* and *S. antibioticus* which produce neither metabolite.

2. Materials and methods

Seed cultures of all Streptomycetes were grown on 75 ml portions of the following, in 200 ml Erlenmeyer flasks: yeast extract, Difco, 10 g; D-glucose, 10 g; distilled water, 1 liter. For experimental cultures, 750 ml portions of the following medium were dispensed into 2.8 liter Fernbach flasks: soy bean meal, 10 g; casamino acids, Difco, 5 g; D-glucose, 20 g; NaCl, 1.33 g; distilled water, 1 liter. Seed cultures were grown for 3 days, experimental cultures for 7 days, at 28°C on a Gyrotary shaker.

Treatment of *S. antibioticus* 3491 with acriflavine, and isolate recovery was as in [4]. With *S. sulfureus* R 6678, amy$^-$ colonies appearing with growth at 37°C were picked, treated with glass beads, and plated out on Hickey and Tresner's agar.

The experimental cultures were steam distilled; two 85 ml distillates were collected, combined, and extracted with three 10 ml portions of methylene chloride. The separated organic layer was dried, filtered, and evaporated to 0.5 ml (N_2 stream, room temp.). The concentrated extracts were analyzed with a Packard gas chromatograph using a column containing 3% OV-17 on 80/100 Gas-Chrom Q (Applied Science Labs.) (6 ft × 2 mm). Temperature was held initially at 100°C for 1.0 min, then increased at 10.0°C/min to a final 10 min hold at 260°C. Actual amounts of metabolites were estimated from peak areas and a calibration graph constructed by use of samples containing known amounts. Radio–gas chromatography used the equipment described in [5]; the column packing and conditions are recorded in the legend to fig.1.

3. Results and discussion

When [2-^{14}C]acetate was added to a culture of *S. antibioticus* 3491, radioactivity was incorporated into both geosmin and 2-methylisoborneol (fig.1A). Similar results were obtained with [1-^{14}C]acetate. The conversion, acetate → geosmin, was also shown in *S. antibioticus* 3720 (which does not form 2-methylisoborneol) and to a lesser degree in *S. sulfureus* R 6678 (grown on a glucose–asparagine minimal medium).

Acetate incorporations could have resulted from either a polyketide or an isoprenoid pathway; since the utilization of a single polyketide chain requires the introduction of an extra methyl group into geosmin,

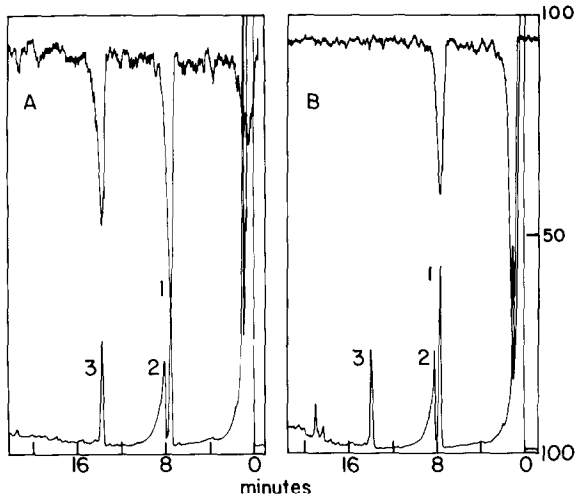

Fig.1. Incorporation of precursors into geosmin and 2-methylisoborneol. Cultures of *S. antibioticus* 3491, grown on soy bean medium for 7 days, were examined by radio–gas chromatography. The column (6 ft × 4 mm) contained 3% SP 2250 on Supelcoport 80/100. Temperature, initially 80°C for 1 min, was increased at 0.5°C/min to a final hold at 300°C. The lower traces are from the flame ionization detector (sensitivity setting of 16×10^{-11} A), the upper from the proportional counter; for both, the ordinate scale is from 0–100% of full scale deflection. (A) A total of 1 mCi [2-^{14}C]acetic acid, sodium salt (spec. act. 47.5 mCi/mmol), was dissolved in 1 ml sterile water; 0.3 ml portions were added to a culture at days 3, 4 and 5 of growth, the remainder, along with washings, being added on day 6. 100% full scale deflection on the proportional counter trace represents 1000 cpm. (B) A total of 250 μCi L-[*methyl*-^{14}C]methionine (spec. act. 80.2 mCi/mmol) was dissolved in 4 ml sterile water. Portions (1 ml) were added to a culture on days 3–6 of growth. 100% full scale deflection on the proportional counter trace represents 2000 cpm. Peak identification: (1) methylisoborneol; (2) unidentified component; (3) geosmin.

utilization of L-[*methyl*-^{14}C]methionine was studied. When *S. antibioticus* 3491 was grown with this precursor, radioactivity was incorporated into 2-methylisoborneol, but not into geosmin (fig.1B).

The incorporation of radioactivity from both acetate and L-[*methyl*-^{14}C]methionine into 2-methylisoborneol strongly suggests that this metabolite derives from a monoterpene (C_{10}) precursor of the isoprenoid pathway, with the 'extra' C-2 methyl group added by methyl transfer from *S*-adenosylmethionine (fig.2). Since isoborneol was not formed in any of our cultures, methylation may be an early event. The postulated origin [6] of 2-methylisoborneol by degradation of a sesquiterpene (C_{15}) is not consistent with our results.

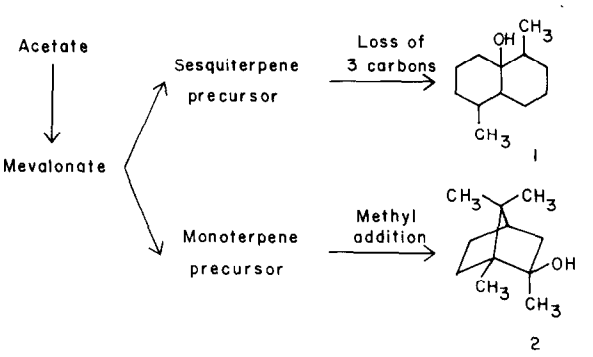

Fig.2. Proposed biosynthetic pathways for geosmin and methylisoborneol.

Geosmin, however, is most likely derived from a sesquiterpenoid precursor (fig.2). Our observations show that neither methyl group of geosmin is derived from methionine – such a utilization would be required if this metabolite were derived from a single, linear, polyketide. A derivation of geosmin from two polyketide chains (without a methylation) cannot be ruled out but seems unlikely; the construction of naphthalenoid and naphthalene related nuclei in this way is a rare event. The most likely C_{15} precursor has the bicyclic eudesmanoid skeleton although a monocyclic germacranoid is possible; removal of an isopropyl side chain is required.

Some Streptomycetes tended to produce rather transparent colonies, lacking the usual aerial mycelium and spores, which resembled the non-differentiating, 'bald' isolated in [7] and the aerial mycelium negative (amy$^-$) isolates in [4]. When *S. sulfureus* R 6678 was grown at 28°C all of the colonies were covered with aerial mycelium and spores, but at 37°C, large numbers of amy$^-$ colonies were present and the pronounced odor associated with growth at 28°C was absent. After growth of a typical amy$^-$ isolate on liquid medium, the steam distillate was devoid of geosmin (the spore-forming colonies produced 1.5 mg geosmin/l, and the gas chromatographic assay would have detected as little as 2 μg/l). These precise analytical results complement the earlier conclusions based solely on lack of odor in amy$^-$ isolates of other Streptomycetes [4]. Identification by odor or lack of odor is unreliable, since geosmin may even be present in culture media without an earthy odor [1].

It was important to determine whether or not the monoterpene derived 2-methylisoborneol was produced by amy$^-$ isolates of *S. antibioticus* 3491 which

normally produces geosmin (0.3 mg/ml and 2-methylisoborneol (0.35 mg/l). Growth at 37°C did not lead to non-differentiated colonies. However, treatment with the intercalating agent, acriflavine [4], gave 15 stable isolates differing in morphology and pigmentation characteristics. When grown in liquid medium all isolates produced essentially the same wet weight of cells as did the spore forming colony. However, these isolates produced neither geosmin nor 2-methylisoborneol (the detection limit for 2-methylisoborneol on gas chromatography was 4 μg/l).

The amy$^-$ isolates clearly are unable to biosynthesize the isoprenoid derived secondary metabolites. In [4] an association between odor production and the presence of a plasmid was favored but the involvement of a transposable genetic element [8] could also be responsible for the results in [4] and those reported here.

Acknowledgements

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