Oxidative Cyclization of Linalol by Various Microorganisms

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($\pm$) Linalol is cyclized into *cis* and *trans* linalol oxides by various microorganisms. This reaction, assuming an intermediate epoxidation step, is analogous to the corresponding step proposed for the biosynthesis of ionophorous antibiotics.

In previous work,¹⁾ we have shown that a model unsaturated alcohol, ($\pm$) linalol **1**, could be cyclized into *cis* and *trans* oxides **2** (Scheme 1) containing a tetrahydrofuran ring by *Streptomyces albus* (NRRL B1865), which synthesizes nigericine.²⁾ The presence in **2** of a C₇ hydroxyl implies intermediate formation of linalol epoxide, which was not isolated.

We report here further work, on this reaction with other microorganisms, undertaken with the aim of establishing whether it is general for those microorganisms known to produce ionophorous antibiotics, and whether it also occurs with microorganisms which do not produce them.

SCHEME 1.

MATERIALS AND METHODS

Products and microorganisms. ($\pm$) Linalol was a commercial product (Fluka). The strains used were: *Streptomyces albus* (NRRL B1865); *Streptomyces hygroscopicus* (NRRL 3444); *Streptomyces cinnamonensis* (ATCC 15413); *Streptomyces griseus* (ATCC 10137); *Beauveria sulfurescens* (ATCC 7159). The other strains used, *Streptomyces albus* (LAB 3840) and *Streptomyces griseus* (LAB 2142), were described in a previous paper.³⁾

Culture conditions. All cultures were grown by the two stage fermentation procedure in different media. Slant cultures were inoculated into 100 ml of seed medium in a

500 ml flask and the flask incubated at 27°C for 72 hr with shaking at 200 rpm. Five milliliters of the resulting vegetative growth were transferred to 500 ml flasks containing 100 ml of a culture medium. The different media used were (in g/liter of tap water).

Medium 1. Dextrose (30), cornsteep solid (5), soybean meal (5) and CaCO₃ (5), pH adjusted to 7.2 by NaOH.

Medium 2. Meat extract (5), Difco casamino acids (5), peptone (5), NaCl (5) and dextrose (10), pH adjusted to 6.8 with NaOH.

Medium 3. Dextrose (20), soybean meal (5), yeast extract (5), K₂HPO₄ (5) and NaCl (5).

Medium 4. Glucose (5), soybean meal (5), yeast extract (2.5), dextrine (20) and CaCO₃ (1), pH adjusted to 7.2 by KOH.

Medium 5. Dextrose (10) and cornsteep solid (12), pH adjusted to 5 by NaOH.

Medium 6. Dextrose (10), yeast extract (1), meat extract (1) and Difco casamino acid (4).

Medium 7. Beet molasses (20), dextrose (10), peptone (5), yeast extract (2) and CaCO₃ (2).

Medium 8. Dextrose (30), soybean meal (25), KCl (0.1), K₂HPO₄ (0.1), MnCl₂·4H₂O (0.6) and FeSO₄·7H₂O (0.3), pH adjusted to 8 by KOH.

Medium 9. Dextrose (40), cornsteep solid (10), FeSO₄·7H₂O (0.5) and CaCO₃ (5), pH adjusted to 7.2 with NaOH.

Medium 10. Saccharose (30), soybean meal (10), peptone (4), K₂HPO₄, K₂HPO₄ (1) and CaCO₃ (5).

Bioconversion conditions. Fermentations were carried out under the same conditions as seed culture. After shaking for 24 hr, ($\pm$) linalol was added at 50 mg/flask. After the fermentation period (Table I), the flasks were filtered, the filtrate saturated with ammonium sulfate extracted three times with ether, and dried. After evaporating the solvent, the residue was analyzed by GPC (stainless steel column, 6ft x 0.125 inch, packed with 20% carbowax on Chromosorb W, with an oven temperature

TABLE I. OXIDATIVE CYCLIZATION OF ($\pm$) LINALOL BY VARIOUS MICROORGANISMS

Microorganism	Culture media ^a	Antibiotic produced	Fermentation time	($\pm$) linalol	Linalol oxides <i>cis</i>	Linalol oxides <i>trans</i>
<i>S. albus</i> sub. sp. NRRL B1865	1~6	Nigéricine	7 days			
Without oleate				95	3	2
With oleate				42	32	26
<i>S. hygrosopicus</i> NRRL 3444	2~7	Dianemycin	7 days			
Without oleate				89	5	6
With oleate				63	19	18
<i>S. cinnamomensis</i> ATCC 15413	4~8	Monensin	7 days			
Without oleate				63	17	19
With oleate				38	28	34
<i>S. albus</i> LAB 3840	1~9	Alborixin	7 days			
Without oleate				35	21	44
With oleate				38	22	35
<i>S. griseus</i> LAB 2142	1~10	Grisorixin	7 days			
Without oleate				90	4	6
With oleate				40	39	21
<i>S. griseus</i> ATCC 10137	3~3		7 days			
Without oleate				20	34	8
With oleate				16	40	38
<i>Baeuveria sulfurescens</i> ATCC 7159	5~5		16 hr	0	44	46

^a First number=seed medium; second number=fermentation medium.

of 120°). Each experiment was conducted with and without methyl oleate (1%). Controls consisted of seeded media without linalol and of linalol added in non-seeded media shaken under the same conditions.

RESULTS AND DISCUSSION

Microorganisms known to synthesize ionophorous antibiotics other than *S. albus* were found to oxidize ($\pm$) linalol, giving mixtures of *cis* and *trans* isomers (Table I). Except for *S. albus* (LAB 3840), it proved necessary to add methyl oleate to the medium to achieve a good yield. The reasons for this remain to be elucidated. Other microorganisms known not to produce ionophorous antibiotics were tested (Table I). *S. griseus* (ATCC 10137) has been used for drug metabolism; the mold *B. sulfurescens* is known to be capable of hydroxylation⁴⁾ and reduction⁵⁾ reactions. Oxidative cyclization of ($\pm$) linalol also occurred with these microorganisms. *B. sulfurescens* gave the best yields (15~20% determined using an internal standard) in the shortest time (16 hr). Linalol oxides were the only products isolated; the rest of the starting material was

probably metabolized by the microorganisms.

An analogous reaction has already been described with a strain of soil *Pseudomonas*⁶⁾ and with a strain of *Botrytis cinerea*.⁷⁾ In the former case, linalol, which was used as a source of carbon, gave a mixture of products including a small of linalol oxide identified by ¹H NMR. In the second example, linalol was identified by chromatography-mass spectrometry. The yields obtained here, although quite low, are adequate to allow the reaction to be used on a preparative scale.

The linalol oxides obtained have a C₇ hydroxyl, implying that the reaction takes place in all likelihood *via* linalol epoxide. This was verified chemically by treating linalol with perphthalic acid.¹⁾

The biosynthesis of carboxylic polyether antibiotics is assumed to involve the formation of tetrahydrofuran rings from an epoxidated polyene precursor.^{8,9)}

This cyclisation reaction observed on ($\pm$) linalol, especially with ionophorous producing microorganisms, agrees with the accepted hypothesis for their biosynthesis and may be

considered as a model for the biosynthesis of those antibiotics.

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