

## SHORT COMMUNICATIONS

### CAMPHOR DEGRADATION BY STRAINS OF *PSEUDOMONAS* AND *MYCOBACTERIUM* ISOLATED FROM SOIL\*

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#### Abstract

Three different isolates of *Pseudomonas* and two of *Mycobacterium* capable of the utilization of camphor as sole carbon source were isolated from soil. Evidence was obtained indicating that the strains of *Pseudomonas* and one of the strains of *Mycobacterium* metabolize camphor via pathways already described for *Ps. putida*. The other strain of *Mycobacterium* appeared to use a different pathway involving the 6-*exo*-hydroxylation of camphor. The disappearance of camphor and the time course of accumulation of intermediates in its degradation is described for one strain of *Pseudomonas*.

#### Introduction

The pathway of camphor degradation shown in Scheme 1 (Conrad 1965*b*) has been established for *Pseudomonas putida* strain PpG<sub>1</sub> and *Pseudomonas* strain C<sub>5</sub> (Bradshaw *et al.* 1959; Conrad *et al.* 1961, 1965*a*; Gunsalus *et al.* 1965; Hedegaard and Gunsalus 1965) and *Ps. testosteroni* (Jacobsen *et al.* 1964). A second different pathway, involving the 6-*exo*-hydroxylation of camphor followed by conversion of the hydroxyl group to a keto group and ring cleavage, has been described for an orange-pigmented diphtheroid strain T<sub>1</sub> (Kay *et al.* 1962; Chapman *et al.* 1963, 1966; Kuo 1965).

The studies described in this report were aimed at examining the types of bacteria capable of using camphor as a carbon source that could be isolated from soil, and the way in which these isolates degraded camphor.

#### Materials and Methods

Enrichment cultures for the isolation of camphor degraders were established by inoculating small soil samples into liquid medium having the following components in grams per litre of distilled water: (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.5; K<sub>2</sub>HPO<sub>4</sub>, 0.1; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.2; Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O, 0.01; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.001; camphor, 1.0. The enrichment cultures were incubated at 30°C until turbidity was visible. After two transfers through fresh liquid medium and subsequent inoculation onto an agar medium of the same composition, pure cultures of camphor utilizers were obtained and characterized. The isolates were identified according to the scheme of Skerman (1967). A culture of *Ps. putida* strain PpG<sub>1</sub> was obtained from Dr. I. C. Gunsalus, University of Illinois, Urbana, USA. An orange-pigmented diphtheroid strain T<sub>1</sub> was obtained as *Mycobacterium rhodochrous* NCIB 9784 from Torry Research Station, Aberdeen, Scotland.

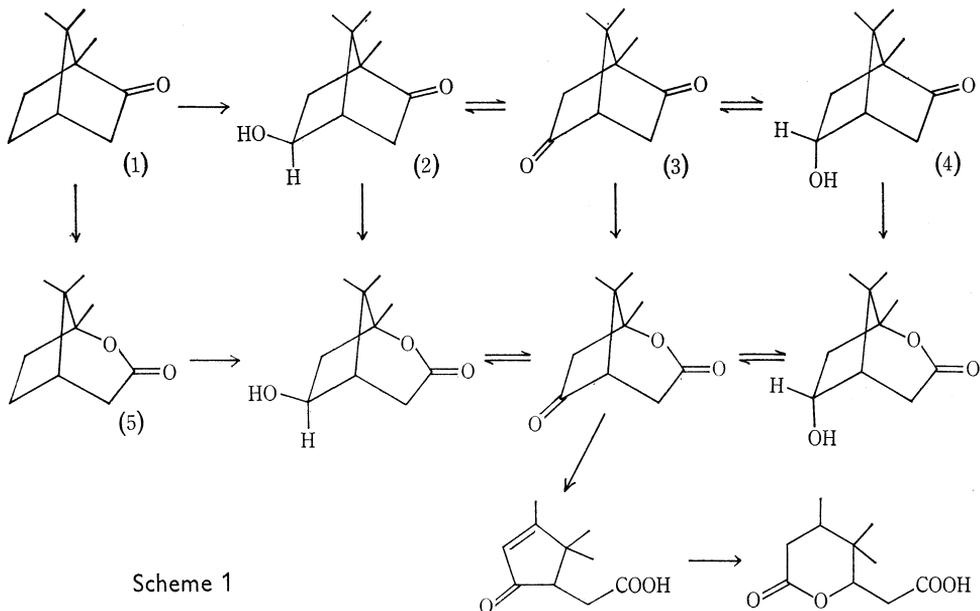
Growth experiments were performed using the medium already described which was sterilized by filtration (mean pore size 0.22 µm; Millipore Corp., Bedford, USA) and dispensed aseptically into 250-ml side-arm flasks in 50-ml amounts. Incubation of the growth flasks was carried out in a

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shaking water-bath at 30°C. A 2% (v/v) inoculum of camphor-adapted cells was used and growth was measured spectrophotometrically at 540 nm.

Chemical analyses of cultures for camphor and intermediates in its degradation were performed by gas chromatography of dichloromethane extracts. Analyses were made using a Shimadzu gas chromatograph model GC4APTF equipped with dual flame ionization detectors. Two pairs of matched 2 m by 2.5 mm stainless steel columns were employed and were packed with either (1) 20% Carbowax 20M terminated with terephthalic acid on Anakrom A 50/60 mesh, or (2) 15% SE-30 on Anakrom 50/60 mesh. Operating conditions for column 1 were carrier gas flow 85.6 ml/min, column temperature 180°C, and for column 2 carrier gas flow 100 ml/min, column temperature 150°C. In both cases the carrier gas was oxygen-free nitrogen. Camphor concentration was estimated using the Carbowax 20M column and employing methyl salicylate as an internal standard.



Column chromatography for metabolite purification was performed by elution from a Florisil 80/100 column with a 50% n-pentane-ether mixture. Preparative gas chromatography, with a Shimadzu GC1C gas chromatograph and a 6.2 m by 17 mm stainless steel column of 20% Carbowax 20M terminated with terephthalic acid on Anakrom A 50/60 mesh was used for further purification. Operating conditions were oxygen-free nitrogen as carrier gas at 240 ml/min, column temperature 180°C. Infrared analysis of purified metabolites and authentic samples was performed with a Beckman IR8 infrared spectrophotometer.

(+)-Camphor (1) was obtained from E. Merck AG, Darmstadt, Germany, and 5-*exo*-hydroxycamphor (2), 2,5-dioxocamphane (3), 5-*endo*-hydroxycamphor (4), and 1,2-campholide (5) were obtained from Dr. I. C. Gunsalus.

### Results

Three isolates of *Pseudomonas* (strains 1, 2, and 3) and two of *Mycobacterium* (strains 1 and 2) capable of utilizing camphor as a sole carbon source were isolated from soil enrichments. *Pseudomonas* strain 3 produces a green fluorescent pigment and appears closely related to *Ps. putida* whereas the other two strains do not produce fluorescent pigments. Colonies of *Mycobacterium* strain 1 were orange and the bacterium shows a similarity to *M. rhodochrous*. The other strain of *Mycobacterium* produced white colonies under similar conditions.

Authentic samples of 2,5-dioxocamphane, 1,2-campholide, 5-*exo*-hydroxycamphor, and 5-*endo*-hydroxycamphor had retention times of 5.6, 9.8, 18.2, and 23.8 respectively when analysed by gas chromatography using the Carbowax 20M column.

Growth curves of each of the isolates growing on 0.1% camphor as the sole carbon source were made and the total disappearance of the substrate was noted in each case. Cultures of *Pseudomonas* strain 1 accumulated three neutral compounds of camphor degradation during growth on this substrate (Table 1) and these were found to have retention times identical with those of compounds (2), (3), and (5). Cultures of *Pseudomonas* strains 2 and 3 accumulated these and compound (4) as well. *Ps. putida* cultures accumulated only (2), (3), and (4) when grown under identical conditions.

TABLE 1  
GAS CHROMATOGRAPHIC DATA FROM ANALYSES OF CULTURE EXTRACTS  
OF CAMPHOR-UTILIZING BACTERIA

Bacterium	Retention times (min) of chromatogram components on	
	Column 1	Column 2
<i>Pseudomonas</i> strain 1	5.6, 9.8, 18.2	
Strain 2	5.6, 9.8, 18.2, 23.8	
Strain 3	5.6, 9.8, 18.2, 23.8	
<i>Mycobacterium</i> strain 1	9.0	6.1
Strain 2	5.6, 9.8	7.1
<i>Ps. putida</i> strain PpG <sub>1</sub>	5.6, 18.2, 23.8	
<i>M. rhodochrous</i> NCIB 9784	9.0	6.1

*Mycobacterium* strain 1 and *M. rhodochrous* NCIB 9784 both accumulated one compound with a retention time of 9.0 min on one column and 6.1 min on the other. *Mycobacterium* strain 2 accumulated two compounds with retention times equal to those of (3) and (5).

The intermediate formed by *Pseudomonas* strain 1 that had a retention time of 9.8 min was purified by column chromatography on Florisil and preparative gas chromatography. Its i.r. spectrum was identical with that of authentic 1,2-campholide (5).

The growth curve of *Pseudomonas* strain 1 related to the disappearance of substrate and the appearance of neutral intermediates of camphor degradation on gas chromatograms of culture extracts is shown in Figure 1.

### Discussion

While most of the evidence obtained for the production of the various intermediates in camphor degradation relies upon the matching of their retention times with those of authentic compounds in gas chromatographic analysis, it is considered sufficient to indicate that the strains of *Pseudomonas* isolated possess the same pathway described for *Ps. putida* strain PpG<sub>1</sub> (Bradshaw *et al.* 1959; Conrad *et al.* 1961, 1965a; Gunsalus *et al.* 1965; Hedegaard and Gunsalus 1965). In the

case of *Pseudomonas* strain 1, it was possible to isolate sufficient material for purification and characterization as 1,2-campholide by i.r. analysis.

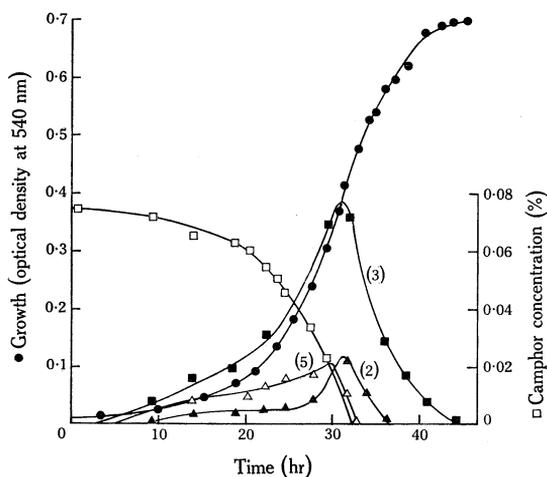


Fig. 1.—Camphor degradation by *Pseudomonas* strain 1; growth of organism and substrate disappearance related to accumulation of products (2), (3), and (5) (shown as a comparison of gas chromatography peak heights).

When the three strains of *Pseudomonas* and the authentic *Ps. putida* were grown under identical conditions there were slight differences in the types of intermediates which accumulated in the cultures, indicating differences in reaction rates.

Because *Mycobacterium* strain 2 cultures accumulated materials having retention times identical with those of authentic samples of 2,5-dioxocamphane and 1,2-campholide, this strain appears to degrade camphor by a mechanism similar to that of *Ps. putida* strain PpG<sub>1</sub>. On the other hand *Mycobacterium* strain 1 appears to degrade camphor in a fashion similar to that of *M. rhodochrous* NCIB 9784.

The sequential appearance and disappearance of intermediates of camphor degradation described for *Pseudomonas* strain 5 by Hedegaard and Gunsalus (1965) was not observed in the present study for *Pseudomonas* strain 1. 2,5-Dioxocamphane was present in the culture medium both before and after the overlapping appearance and disappearance of 5-*exo*-hydroxycamphor and 1,2-campholide (Fig. 1). These differences are understandable because of the complex and branched nature of the pathway of camphor degradation. Slight differences in cultural conditions such as extent of oxygenation of the culture could have an effect upon the course taken during degradation.

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