Intermediates in the Bacterial Degradation Pathway of α -Terpineol[†]

Kohji Tadasa

Department of Agricultural Chemistry, College of Agriculture, University of Shinshu, Ina City, Nagano-ken 396, Japan Received May 30, 1977

To elucidate the mechanism of biosynthetic route of terpenes, many workers have investigated. The author, as one of those studies, has reported that α -terpineol was assimilated by a soil bacterium, tentatively identified as Pseudomonas aeruginosa, and was converted to borneol and terpinolene as the neutral catabolic products.²⁾ Borneol, one of them, has been supposed to be produced via a-terpineol as an intermediate in a biosynthetic pathway by Banthorpe et al.3) The conversion of a-terpineol to borneol involves an interesting cyclization process from the monocyclic (cyclohexene) to the bicyclic (bornane) skeleton. A hypothetical intermediate, terpinylcation (I) has been supposed to exist on the way to bornane skeleton.⁴⁾ Some supports were offered for the hypothesis in the previous paper.2)

Borneol was obtained in a large amount in comparison with other small amount products during the fermentation of α -terpineol. It was thought as an end-product, because borneol was not assimilated further by the strain as a carbon source. After many times of cultivation, however, the strain obtained the ability of assimilation for borneol, and produced several neutral metabolites. Though those metabolites have already been identified by Hayashi *et al.*, it was newly demonstrated that the path to those metabolites involved α -terpineol as an intermediates.

Moreover, an acidic product, which was handly been produced when this strain was used at first was formed from α -terpineol. The present communication deals with the isolation and identification of those metabolites and it is reported that borneol and the acidic product are intermediates in the degradation pathway of α -terpineol.

The medium and the method of cultivation were the same as described in the early report.²⁾ The acidic product in the ether extract was isolated by extraction with 5% NaHCO₃ solution and reextraction with ether after acidification (pH 2). The acidic product was recrystallized several times from ethanol and water. It was colorless crystal, soluble in alcohol, not

[†] This paper is Part III of α -terpineol degradation. For Parts I and II, see references 1, 2).



FIG. 1. Oxygen Uptake with Each Metabolite from α -Terpineol by α -Terpineol-grown Cells (a) and Borneol-grown Cells (b) of *Pseudomonas aeruginosa*.

The side-arm contained 0.3 ml of 50 mM substrate in 1/10 M phosphate buffer (pH 7.2). The center well contained 0.3 ml of 20% KOH and filter paper. The cells in the main compartment (*ca.* 1.2 mg, dry wt.) were added into each vessel after suspended in 2 ml of 1/10 M phosphate buffer (pH 7.2).

 \bigcirc — \bigcirc , α -terpineol; \times — \times , oleuropeic acid; \triangle — \triangle , borneol; \bigcirc — \bigcirc , endogenous.

The reaction was held at 30° C, and was started after 30 min equilibration at same temperature.



FIG. 2. Time-course Change of Acid Product, Cells and pH.

 \bigcirc — \bigcirc , cells; ×—×, pH; \triangle — \triangle , acid product.

in ether and chroloform. It gave a single spot on thin-layer chromatogram with following solvent: benzene-dioxane-acetic acid (90: 45: 4, v/v), Rf 0.75. It was characterized by melting point: 154~156°C, $[\alpha]_{D}^{17.8}$: +19.4° (c=2.5, ethanol) and m/e: 184 (M⁺), 166 (M⁺-H₂O). The IR spectrum, the NMR spectrum of the methyl ester and the UV spectrum ($\lambda_{max} =$ 217 nm) are in agreement with those of oleuropeic acid (II).^{5, 8)} This product from α -terpineol used ($[\alpha]_{D}^{18.3}$: -11.8° , ethanol) will be a complex of (+) and (-). Oleuropeic acid has been obtained by Shukla et al. as a product from α and β -pinenes in a soil pseudomonad.⁵⁾ They proposed that oleuropeic acid from pinenes was produced through a-terpineol as a precursor and not degraded any more (end-product). When the oleuropeic acid, which was obtained as a degradation product from a-terpineol, however, was used as the sole source of carbon and energy, it was well assimilated by the same strain and was shown to be one of the intermediates during the degradation of a-terpineol.

(₩)

Figure 1 shows oxygen uptake for each metabolite by a-terpineol-grown cells and borneol-grown cells. Figure 2 shows the changes of the quantity of oleuropeic acid, cells and pH to time. The acidic product is formed relatively slow from the initiation of cultivation.

With the assimilation for borneol of this strain, two substances were confirmed as neutral product. One of the major products (solvent system for TLC; hexane-ether (15:13, v/v), Rf 0.46) was completely in agreement with authentic campholytolactone (III), as reported,⁷⁾ in Rf value and IR spectrum. Rf value on TLC of the other minor product was in agreement with that of 6-hydroxycampholytolactone (IV).8) These were suggested to be the intermediates in the degradation pathway of a-terpineol as well as borneol, Above two products the third substance appeared on TLC, which is supposed to be camphor by the same Rf value. Shukla et al. have proposed that borneol and a-terpineol were produced via two different pathways from pinenes; Borneol was formed without ring rupture of the cyclobutane in the bicyclo skeleton, while a-terpineol was formed with complete ring rupture of the cyclobutane in the bicyclo skeleton. The results in the present study, however, offer possibility of one path from α -terpineol to borneol.

These are shown in the above scheme.

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