

Microbiological Transformations of Terpenes

III. Transformations of Some Mono- and Sesqui-Terpenes

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ABSTRACT

PREMA, B. R. (National Chemical Laboratory, Poona, India) AND P. K. BHATTACHARYYA. Microbiological transformations of terpenes. III. Microbiological transformations of some mono- and sesqui-terpenes. *Appl. Microbiol.* **10**:529-531. 1962.—The action of *Aspergillus niger* on mono- and sesqui-terpenic hydrocarbons, such as carane, 3-carene, α -santalene, and humulene was studied in shake cultures. Carane, Δ^3 -carene, and humulene proved to be rather resistant to oxygenation by the experimental strain of *A. niger*. Carene yielded a hydroxyketone, $C_{10}H_{14}O_2$, in poor yields after prolonged fermentation. The sesqui-terpene hydrocarbon, α -santalene, was degraded mainly to an acid, *tere*-santalic acid. Two hydroxylated products were also obtained from α -santalene, viz., *tere*-santalol and an alcohol, $C_{15}H_{24}O$.

In previous communications (Bhattacharyya et al., 1960; Prema and Bhattacharyya, 1962), the stereospecific conversion of α -pinene into oxygenated products such as *cis*-verbenol, verbenone, and *trans*-sobrerol by a strain of *Aspergillus niger* was described. It was considered necessary to determine whether α -pinene alone is uniquely oxygenated microbiologically, or whether such transformations are also possible with other terpenoid hydrocarbons. This communication deals with studies on the action of the experimental strain of *A. niger* (612) on carane (I), Δ^3 -carene (II), α -santalene (III), and humulene (IVa). The roman numerals refer to the structural formulas shown in Fig. 1.

MATERIALS AND METHODS

All melting and boiling points are uncorrected. The rotation was recorded in chloroform, unless otherwise specified, with a 1-dm tube. The infrared spectra were recorded in Nujol mull or as a liquid film with either a Grubb-Parsons double-beam spectrophotometer or a Perkin-Elmer Infracord.

Carane, Δ^3 -carene, humulene, α -santalene, and *tere*-santalic acid were obtained through the courtesy of S. C. Bhattacharyya, Essential Oils Division of this Laboratory, and had the following physical constants: carane, bp 168 to 170 C, $[\alpha]_D = -10.8^\circ$; carene, bp 168 to 169 C, $[\alpha]_D = +11.5^\circ$; α -santalene, bp 121 to 123 C/10 mm,

$[\alpha]_D = +10.8^\circ$; humulene, bp 113 to 115 C/6 mm; *tere*-santalic acid, mp 155 to 157 C, $[\alpha]_D = -77^\circ$ (benzene).

Fermentation. The mold, conditions for fermentation, and the method of extraction were essentially the same as that described for the transformation products of α -pinene (Prema and Bhattacharyya, 1962). The *n*-butanol extraction was omitted since all the metabolites from the terpenes were extractable in ether.

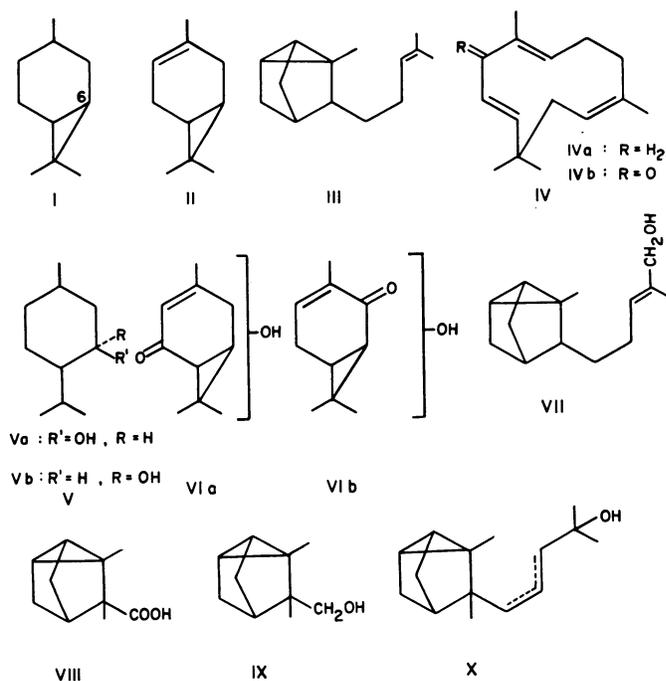


FIG. 1. Compounds investigated: I, carane; II, Δ^3 -carene; III, α -santalene; IVa, humulene; IVb, zerumbone; Va, menthol; Vb, isomenthol; VIa, b, hydroxyketone of Δ^3 -carene; VII, α -santalol; VIII, *tere*-santalic acid; IX, *tere*-santalol; X, intermediate oxidation product.

RESULTS

Carane and Δ^3 -carene. These hydrocarbons were added in concentrations of 0.5% (v/v) to a 30-hr growth of the mold. The fermentation was allowed to continue for 72 hr.

The neutral ether extracts from 40 shake flasks were subjected to fractional distillation or partition between *n*-hexane and 90% ethanol. In the case of carane, the hydrocarbon was recovered (3.3 to 4.2 g) by fractional

distillation, leaving a small amount of lipid residue. The infrared spectrum of the distillate corresponded to that of pure carane and did not exhibit any trace of hydroxyl or carbonyl absorption.

In the case of carene, besides the unreacted hydrocarbon (2 to 3 g) a viscous high-boiling distillate (VI; 0 to 300 mg) was obtained both on fractional distillation and from the alcoholic layer after partition between hexane and 95% ethanol: bp 80 to 84 C/9 mm; infrared $\bar{\nu}_{\max}$ 3350 cm^{-1} (OH), 1659, 1610 cm^{-1} (α, β -unsaturated ketone), $\bar{\nu}_{\max}$ 1033, 990 cm^{-1} (cyclopropane ring); λ_{\max} 263, $\log \epsilon$ 3.82. Found: C, 72.01; H, 8.57. $\text{C}_{10}\text{H}_{14}\text{O}_2$ requires: C, 72.26; H, 8.49).

α -Santalene (III). This hydrocarbon was added to 40 shake flasks containing 30-hr cultures at a level of 0.2% (v/v), and the fermentation was allowed to continue for 24 hr. The cultures were extracted with ether in the manner described (Prema and Bhattacharyya, 1962), and the ether extract was separated into acidic and neutral components.

The acidic component was recovered in chloroform from the aqueous sodium carbonate extracts after acidification. The crystalline residue (825 mg), after removal of chloroform, was purified by repeated sublimation at 140 to 145 C under the vacuum of aspirator pump (30 mm; yield: 705 mg): mp 152 to 154 C; $[\alpha]_{\text{D}} = -73.7^\circ$ (C, 3.37 benzene). Literature (Semmler and Bode, 1907): mp 154 to 156 C; $[\alpha]_{\text{D}} = -77^\circ$ for *tere-santalol*. Infrared $\bar{\nu}_{\max}$ 2660 cm^{-1} (bonded carboxyl OH), 1,760 cm^{-1} (C = O of carbonyl) 880, 835 cm^{-1} (tricyclene), neutral equivalent 161 \pm 3. Found: C, 71.86; H, 8.85. Calculated for $\text{C}_{10}\text{H}_{14}\text{O}_2$: C, 72.26; H, 8.49). Mixed mp 153 C.

The neutral extractives from ether (4.15 to 4.60 g) were chromatographed over neutral alumina (grade II, 175 g). The column was developed with graded solvent mixtures of *n*-hexane, benzene, and ether. *n*-Hexane eluted unreacted α -santalene (III; 3.1 to 3.6 g) and benzene; ether mixtures eluted a solid alcohol, IX (25 to 40 mg), and a liquid alcohol, X (15 to 100 mg): bp 140 to 145 C/6 mm; infrared $\bar{\nu}_{\max}$ 3,400 (OH), 1660, 980 (*trans*-disubstituted double bond), and 880, 855 cm^{-1} (tricycline ring system). Recovered unchanged after treatment with acetic anhydride and pyridine and with chromium trioxide and pyridine.

The solid alcohol, IX, was purified by repeated sublimation at 90 to 95 C under the vacuum of an aspirator pump (30 to 35 mm) to the constant mp 108 to 110 C; $[\alpha]_{\text{D}} = +10.5^\circ$ (c, 2.01). Literature (Semmler and Bode, 1907), mp 112 to 114 C; $[\alpha]_{\text{D}} = +12^\circ$. Infrared for *tere-santalol* $\bar{\nu}_{\max}$ 3,300 cm^{-1} (OH), 882, 861 cm^{-1} (tricyclene).

Lithium aluminium hydride reduction of tere-santalol acid. Compound VIII (150 mg) was reduced with 100 mg of lithium aluminium hydride in 10 ml of ether with stirring under reflux for 2 hr. The complex was decomposed first with water and then with 2 N sulfuric acid. The ether layer after drying and evaporation yielded *tere-*

santalol (120 mg). Purified by sublimation, mp 110 to 112 C; mixed mp with compound IX, 108 to 110 C. The infrared spectra of *tere-santalol* and compound IX were identical.

The liquid alcohol, X, was purified by distillation under reduced pressure (bp 140 to 145 C, bath temp/6 mm); infrared $\bar{\nu}_{\max}$ 3,400 cm^{-1} (OH), 1660 cm^{-1} (double bond), 980 cm^{-1} , (*trans*-disubstituted double bond), 850, 855 cm^{-1} (tricyclene). Found: C, 81.24; H, 11.29. $\text{C}_{15}\text{H}_{24}\text{O}$ requires: C, 81.75; H, 10.98.

Humulene (IVa): Humulene was added at a level of 0.2% to 40 flasks of a 30-hr-old shake culture of *A. niger*. The fermentation was allowed to proceed for 48 hr. The neutral ether extract (7.1 g) was chromatographed over neutral alumina (grade II, 200 g). The column was developed with petroleum ether (60–80), benzene, and chloroform. Petroleum ether eluted unreacted humulene (6.28 g). The benzene-chloroform mixture eluted a solid alcohol (32 mg). Recrystallized from petroleum ether (60–80): mp 140 to 143 C. Infrared $\bar{\nu}_{\max}$ 3,450 cm^{-1} (OH). Found: C, 81.13; H, 10.47. $\text{C}_{15}\text{H}_{24}\text{O}$ requires: C, 81.76, H, 10.98.

DISCUSSION

The studies on carane (I) and carene (II) were undertaken with the objective that a biological hydroxylation of these compounds at position 6 along with a ring opening could possibly establish a convenient synthesis of menthol (Va) or isomenthol (Vb). And since allylic oxygenation was a predominant metabolic activity of *A. niger* on α -pinene (Prema and Bhattacharyya, 1962), studies were undertaken with α -santalene (III) and humulene (IVa; Sukh Dev, 1960) to explore possible microbiological pathways to α -santalol (VII; Guha and Bhattacharyya, 1944) and zerumbone (IVb; Balkrishnan, Razdan, and Bhattacharyya, 1956).

Compared with α -pinene, carane (I), Δ^3 -carene (II) and humulene (IV) were found to be resistant to oxygenation by *A. niger*, although they are readily susceptible to autoxidation. Carane was recovered unchanged even after 72 hr of fermentation. Δ^3 -Carene, however, yielded in small and variable yields (0 to 1%) of hydroxyketone, $\text{C}_{10}\text{H}_{14}\text{O}_2$ (VI). The ultraviolet and infrared spectra of VI indicated the presence of an α, β -unsaturated carenone system with the cyclopropane ring intact. Two types of structure, VIa or VIb, are admissible for this compound. Further work could not be carried out since the material was unstable; from some batches, none of it could be isolated.

A crystalline alcohol, $\text{C}_{15}\text{H}_{24}\text{O}$, was isolated after fermentation of humulene (IVa). This is probably an artifact, since even in the controls run without the mold traces of a similar product could be detected.

Incubation of α -santalene with *A. niger* resulted in the formation of appreciable amounts of oxygenated products. The solid acidic fraction yielded, after repeated sublima-

tion, *tere*-santallic acid, VIII (12 to 15%), which was identified through its physicochemical properties and by a comparison of the infrared spectra of the mold metabolite with an authentic sample.

The neutral fractions from the fermentation yielded two products on chromatography over alumina: *tere*-santalol (IX) and a liquid alcohol (X), $C_{15}H_{24}O$. The latter product, which was obtained in extremely variable yields, indicated the presence of a hydroxyl group ($3,400\text{ cm}^{-1}$), a *trans*-disubstituted double bond ($1,660, 980\text{ cm}^{-1}$), and a tricyclene system ($855, 880\text{ cm}^{-1}$) in the infrared spectrum. Even though analytical data for X were in good agreement with those of α -santalol (VII), the infrared spectra of the two compounds showed marked differences in the finger print region.

Furthermore, compound X resisted acetylation with pyridine and acetic anhydride, or oxidation with chromium trioxide in pyridine, indicating that the hydroxyl group was tertiary in nature. From this behavior, the tentative structure X was assigned to the compound. The hydroxylation probably takes place on the terminal double bond of α -santalene. An intermediate such as X probably undergoes further oxidation to give rise to *tere*-santallic acid and *tere*-santalol.

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