THE MICROBIOLOGICAL TRANSFORMATION OF FENCHONE[1]

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Received May 27, 1965

In the field of terpene and steroid biochemistry there are now recorded several examples of the microbiological conversion of cyclic ketones to the corresponding lactones, a conversion formally analogous to the Baeyer-Villiger (1899) reactions. The earliest accounts of such transformations were those of Fried et al. (1953) and Peterson et al. (1953), who found progesterone and 17-α-hydroxyprogesterone converted to testololactone by Aspergillus and Penicillium species. Bradshaw et al. (1959) and Conrad et al. (1961a) found that reactions of this type played a part in the metabolism of (+)-camphor by Pseudomonas putida[3] and more recently Laskin et al. (1964a, 1964b) have presumptive evidence for a similar transformation of the A ring of eburicoic acid by Glomerella fusaroides. Cell-free enzyme preparations have been described which catalyze the lactonization of camphor and 2,5-bornanedione respectively to the lactones of 4-hydroxy-4,5,5-trimethylcyclopentane-1-acetic acid and to its 2-oxo-derivative (Conrad et al., 1961b) and of testosterone to testololactone (Prairie and Talalay, 1963).

The following reports the conversion of fenchone (I) to a mixture of 1,2- and 2,3-fencholides (II and III) (Figure 1) through the agency of a Corynebacterium sp., an organism which grows at the expense of either

[1]This work was supported in part by Grant from the National Science Foundation, No. G-24037.

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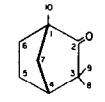
[3]Name assigned by R. Y. Stanier and N. J. Palleroni, University of California, Berkeley, on bases of gelatin liquification -, egg yolk test -, growth: 40° -, 4° -, mannitol -, creatine +, hippuric acid +, trigonellin +.

(+)- or (-)-camphor (Chapman et al., 1963).

d-,l-Fenchone (K & K Laboratories, Plainview, New York) was distilled through a 24 inch Vigreux column at 190-191.5° and chromatographed on Florisil. Ether-light petroleum (2:98 v/v) eluted fenchone free of



d-FENCHONE

I

1,2-FENCHOLIDE

II

2,3-FENCHOLIDE

III

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impurities and after a second distillation the material had $[\alpha]_D^{23}$ + 5.1° in 1,4-dioxane (C,2) indicating nearly an 8% excess of the (+)-enantiomer (based upon $[\alpha]_D^{20}$ + 67° for d-fenchone) (Simonsen, 1949). Corynebacterium sp., strain T_1, was inoculated from stock culture slants into 100 ml sterile nutrient broth (Difco; 8.0 g/liter) and grown at 30° with shaking for 24 hours. Ten ml portions were used to inoculate six 2 liter Erlenmeyer flasks each containing 400 ml of sterile broth. After 24 hours incubation 2.4 g fenchone in N,N,-dimethyl formamide (0.25 g/ml) was equally divided among all six flasks and incubation continued for a further 24 hours. The cells were then centrifuged and the clear neutral supernatant extracted 3 times with 0.5 vol. diethyl ether. The ether solution, after drying over anhydrous Na_2SO_4, was taken to dryness and 1.12 g of pale yellow crystalline solid obtained. When crystallized from light petroleum (b.p. range 60-68°) at -72°, the material had m.p. 73-75°, and $\lambda_{max}^{CHCl_3}$ 5.48μ (Found: C, 70.82; H, 9.49. $C_{10}H_{16}O_2$ requires C, 71.4; H, 9.52).

Thin layer chromatography on silica gel G Brinkmann with ether-light petroleum (40:60, v/v) as solvent, revealed a single component reacting with alkaline hydroxylamine and detectable as its purple ferric hydroxamate. Gas chromatography on a stationary phase of 5% silicone

gum rubber SE-30 at 150° gave only a single peak but on 5% butanediol succinate polyester at 150° a second component, approximately 10% of the total peak area, was distinguishable. A mixture of authentic 1,2- and 2,3-fencholides (II and III)[4], obtained by the Baeyer-Villiger oxidation of fenchone with peracetic acid, showed essentially the same chromatographic properties as the biologically derived material (Table 1). It will be noted that the major component corresponds to the 1,2-fencholide.

Table I. Chromatographic Properties of Fenchone and Related Compounds

Compound	R_f*	Retention time+ on SE-30	Retention time+ on Butanediol succinate polyester
Fenchone (I)	--	0.72	--
1,2-Fencholide (II)	0.34	1.92	8.16
2,3-Fencholide (III)	0.34	1.92	9.40
Major product	0.34	1.92	8.16
Minor product	0.34	1.92	9.40

*Thin layer chromatography on silica gel plates activated at 110° for 30 minutes and developed with ether-light petroleum (40:60 v/v).
+Vapor phase chromatography retention times in minutes after emergence of solvent. Both SE-30 and butanediol succinate polyester were maintained at 150°.

Further support for this assignment was obtained by nuclear magnetic resonance studies. The spectrum of the biological product in $CDCl_3$ was almost identical with that of the synthetic 1,2-fencholide

[4]We would like to thank Dr. R. R. Sauers for providing a mixture of 1,2- and 2,3-fencholides and also for details of the NMR spectra of the separate lactones.

(II), showing two bands at 8.81 and 8.76τ due to the gem-dimethyl and a single resonance at 8.53τ due to the shielded angular methyl. Two weak bands at 8.65 and 8.62τ were also present corresponding to the gem-dimethyl group of the minor component, 2,3-fencholide.

It is of interest that the biological lactonization of fenchone observed here gives both possible lactones. Fenchone very likely induces an enzymatic lactonization system favoring a 1,2- attack but lacking absolute specificity. The spatial arrangement of the methyl groups carried on carbons 1 and 3 thus appears to be sufficiently similar to permit a slow attack at the 2,3-position. The observed proportions of the 1,2- and 2,3-lactones (90:10) contrasts with the chemical oxidation of fenchone with peracetic acid (Sauers and Ahearn, 1961) where 2,3-fencholide is the major product in a 40:60 mixture. Lactonization of fenchone is all the more unusual in view of the findings that camphor, a structurally similar terpene, is degraded by an initial 6-endo-hydroxylation by this organism. In what may be a parallel case, Conrad et al. (1965) have shown a broad specificity range for the purified ketolactonase from Pseudomonas putida, strain Cl. These observations suggest a degree of specificity for both enzyme-inducer and substrate which is not yet fully understood.

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