Tetrahedron Letters No 10, pp. 561-565, 1965. Pergamon Press Ltd.
Printed in Great Britain.

LACTONE INTERMEDIATES IN THE MICROBIAL OXIDATION OF (+)-CAMPHOR (1)

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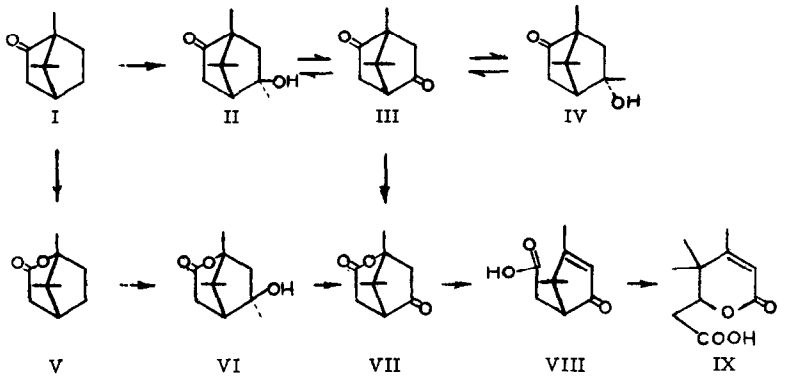
(Received 15 January 1965)

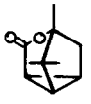
The importance of enzymatic oxidation reactions at carbon in the terpene and steroid families has prompted an investigation of the microbial degradation of (+)-camphor as a model for the biological oxidation and degradation of carbocyclic structures. Previous communications (1,2) have described the oxidation of (+)-camphor by a pseudomonad, strain C1 (previously referred to as strain P), via compounds II, III, IV, VIII, and IX with the implication of VII as an intermediate too unstable to accumulate in the conversion of III to VIII. The conversion of I to IX proceeds by a reaction sequence involving hydroxylation, dehydrogenation, and two lactonizations. Thus, the process for cleavage of both carbocyclic rings of (+)-camphor



involves reactions quite analogous to those found in other instances of microbial degradation (4, 5).

A second pseudomonad, strain C5, isolated from soil by enrichment, has now been found to grow similarly on (+)-camphor in a mineral salts medium with the accumulation of a new series of intermediates leading also to the lactonic acid IX. Extraction of the broths with methylene chloride at appropriate stages during growth and chromatography of the neutral fraction on Florisil yielded two new products. The first (C, 71.64; H, 9.30) was identified as 1,2-campholide (V) by comparison with the synthetic compound obtained by oxidation of d-camphor with 40% peracetic acid buffered with sodium acetate (6). The melting points (168-70°), IR spectra (carbonyl absorption, 5.72μ), n.m.r. spectra and vapor phase chromatographic behavior of the natural and synthetic products were identical.

The second metabolite, m.p. 194-195° (C, 65.02; H, 8.65), was identified as 5-exo-hydroxy-1,2-campholide (VI) by the following data. Infrared analysis ($CHCl_3$) revealed hydroxyl absorption at 2.70 and 2.83μ and δ-lactone carbonyl absorption at 5.81μ. The n.m.r. (7) spectrum in $CDCl_3$ showed a single carbinol proton 4.12 ppm.(quartet), three methyl groups 1.31, 1.26 and 1.03 ppm. (each a singlet) and multiplets between 1.5 and 3.1 ppm. Reaction of this hydroxy lactone with p-toluenesulfonyl chloride-pyridine afforded a mono p-toluenesulfonate derivative which underwent elimination with potassium t-butoxide in t-butyl alcohol to form the saturated tricyclic lactone, X, m.p. 182.5-184.5°, carbonyl absorption at 5.81μ (in $CHCl_3$) but neither olefinic nor hydroxyl absorption in the infrared, n.m.r. peaks (7) at 0.99 ppm. ($C(CH_3)_2$, doublet), 1.23 ppm. (one CH_3, singlet) with a five-proton multiplet at 1.57-2.28 ppm. but no peaks due to



X

olefinic protons, no coloration with tetranitromethane: found: C, 72.41; H, 8.56. Oxidation of VI with ruthenium tetroxide in Freon 11 (8) gave the keto lactone VII, m.p. 164-165.5°, infrared absorption in $CHCl_3$ at 5.69μ (cyclopentanone) and 5.77μ (δ-lactone), found: C, 65.78; H, 7.82; this keto lactone is converted rapidly above pH 9 in aqueous solution to the salt of VIII.

The steric configuration of the hydroxyl group in VI, indicated as exo by the chemical-n.m.r. data (2), was confirmed using purified

DPN-linked dehydrogenases from strain Cl which are specific for II or IV (9). The II dehydrogenase catalyzed dehydrogenation of VI at ca. 0.3 the rate with II while the IV dehydrogenase had no activity. Extracts of strain C5 also contained DPN-linked dehydrogenase activity for VI. The product accumulating after the enzymatic dehydrogenation of VI was VIII, identified by its characteristic absorbance at 235 mμ (E=15,000 cm^2/mmole) and by VPC analysis of its methyl ester on a 4 foot SE30 column (5% on chromsorb W) at 150°.

These data indicate that strains Cl and C5 accumulate camphor intermediates on diverging pathways, which differ only in their sequence of hydroxylation, dehydrogenation, and lactonization and imply that VII is a common intermediate in the metabolic conversions of III and VI to VIII (3). To test this hypothesis the enzymatic conversion of the synthetic keto lactone (VII) to VIII was measured. This conversion was catalyzed by a cell extract of strain Cl and by purified lactonizing enzyme (specific activity--0.63 units/mg protein (3); 1 unit = 1 μmole product formed/min.) at rates of 1.45 and 13.1 μmoles/min./mg. protein respectively. Thus, all data are consistent with the proposal that VII is a common intermediate in both metabolic sequences.

Accumulation in a microbial growth medium of metabolites formed directly from a compound used as the sole carbon and energy source is usually taken as evidence that such metabolites are intermediates on the major (or only) degradative pathway for the carbon source. In our studies, therefore, one might conclude that strain Cl degrades (+)-camphor primarily via compounds II and III, while strain C5 uses the alternate pathway via compounds V and VI. However, in our examination of the enzymic activities in cell extracts from strain Cl and C5 (3, 9-11) we have found that both strains have the full enzyme complement for both pathways. Therefore, when strain Cl grows

on camphor, it is possible that the largest fraction of the total camphor utilized is metabolized rapidly via compounds V and VI with no accumulation of these intermediates and that accumulation of II, III and IV in the medium reflects a relatively slow rate of camphor metabolism by this pathway. Analogous reasoning applies to the metabolism of camphor by strain C5. The accumulation of intermediates in the growth medium is a function of the levels, specificities, K_m's and V_{max}'s of the enzymes induced in the organism by the growth substrate. The comparison of these parameters in strain C1 and C5 will be discussed elsewhere.

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