JOURNAL OF BACTERIOLOGY, Apr. 1970, p. 281-282
Copyright © 1970 American Society for Microbiology
Vol. 102, No. 1
Printed in U.S.A.

Subterminal Oxidation of Aliphatic Hydrocarbons

F. W. FORNEY[1] AND A. J. MARKOVETZ
Department of Microbiology, University of Iowa, Iowa City, Iowa 52240

Received for publication 24 December 1969

Evidence is presented for a catabolic pathway of *n*-alkane oxidation which proceeds via subterminal oxidation rather than methyl group oxidation.

Methyl ketones and secondary alcohols have been implicated as intermediates in the microbial oxidation of aliphatic hydrocarbons. Lukins and Foster (8) showed that acetone, butan-2-one, pentan-2-one, and hexan-2-one were produced from the respective *n*-alkanes by *Mycobacterium smegmatis*. Tetradecan-2-ol was detected by Markovetz, Klug, and Forney (10) when tetradec-1-ene was oxidized by *Pseudomonas aeruginosa*. Fredricks (5) identified decan-2-, 3-, 4-, and 5-one, together with the corresponding secondary alcohols, from the oxidation of *n*-decane by *P. aeruginosa:* the methyl ketone, decan-2-one, was produced in highest concentration. Klein and co-workers (6, 7) demonstrated that an *Arthrobacter* species can transform decane, dodecane, tetradecane, and hexadecane into their respective 2-, 3-, and 4-ketones and matching secondary alcohols. Again, methyl ketones were formed preferentially. These products arose during "co-oxidation" of the hydrocarbon substrates, which would not, by themselves, serve as sole carbon and energy sources. Vestal and Perry (11), using a *Brevibacterium* species in isotope competition experiments, recovered acetone as an intermediate of propane oxidation. The next oxidative intermediate was postulated to be acetol, although none was isolated.

Recently we demonstrated a pathway for degradation of the long-chain methyl ketone, tridecan-2-one, with cells and cell-free extracts of *P. multivorans* and *P. aeruginosa* (2-4). This pathway proceeds through an ester intermediate, undecyl acetate, which is cleaved to acetate and undecan-1-ol:

$$CH_3-(CH_2)_9-CH_2-\underset{\underset{O}{\|}}{C}-CH_3 \rightarrow$$

$$CH_3-(CH_2)_9-CH_2-O-\underset{\underset{O}{\|}}{C}-CH_3 \rightarrow$$

$$CH_3-(CH_2)_9-CH_2-OH + CH_3-COOH$$

[1] Present address: Department of Microbiology, Louisiana State University, Baton Rouge, La. 70803.

Oxidation of the secondary alcohol, tridecan-2-ol, proceeds in a similar manner, i.e., to the ketone, the ester, etc. After elucidation of a catabolic pathway for these two compounds, we endeavored to ascertain whether this pathway operated when the corresponding hydrocarbon, *n*-tridecane, served as substrate. Our results are the subject of this report.

P. aeruginosa strain Sol 20, used in the previous studies (2, 3), was grown in a basal-salts medium (1) containing 0.5% *n*-tridecane which had been freed of oxygenated impurities by passage through a column containing adsorption alumina, 80/100 mesh. After 24 hr, the cells were harvested, resuspended in the 0.5% hydrocarbon medium, and shaken for 12 hr. Culture fluid, freed of cells, was acidified and extracted with diethyl ether. Analysis of the ether extract was performed by gas-liquid chromatography as described elsewhere (2).

Results of gas-liquid chromatography analysis are given in Table 1. Two alcohols were detected: tridecan-2-ol and undecan-1-ol. It is evident from previous work cited above that, if the subterminal catabolic pathway functions, it will generate a secondary alcohol or methyl ketone of substrate chain length and a primary alcohol shorter by two carbon atoms. Although the methyl ketone

TABLE 1. *Gas-liquid chromatography data on alcohols from the oxidation of n-tridecane*[a]

Alcohol	Standard compound	Biological compound
Undecan-1-ol	23.2	23.1
Tridecan-2-ol	27.0	26.9
Undecan-1-ol-TMS[b]	6.8	6.8
Tridecan-2-ol-TMS[b]	8.5	8.5

[a] Retention time in minutes obtained on a 15-ft (4.6 m) column containing 15% FFAP (Varian-Aerograph, Walnut Creek, Calif.) on 100/120 mesh Chromosorb G. Column temperature was 170 C at a flow rate of 50 ml/min.

[b] Trimethylsilyl derivative of the alcohol.

and ester intermediates were not apparent in our analyses, the presence of two alcohols which satisfy these requirements indicates that the same pathway is operative when *n*-tridecane serves as growth substrate.

The degradative pathway described may be applicable also to unsaturated aliphatic hydrocarbons. We have observed that *P. aeruginosa* was able to transform not only tetradec-1-ene into tetradecan-2-ol during growth on the alkene (10), but also tetradecan-2-ol into dodecan-1-ol when the secondary alcohol served as sole carbon for growth (Markovetz and Forney, *unpublished results*).

Methyl group oxidation of both saturated and unsaturated aliphatic hydrocarbons also is accomplished by pseudomonads (9, 10). The relative importance of terminal methyl group, as compared to subterminal methylene group oxidation, remains to be determined.

This investigation was supported by grant GB-6875 from the National Science Foundation.

LITERATURE CITED

1. Dworkin, M., and J. W. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75:592–603.
2. Forney, F. W., and A. J. Markovetz. 1968. Oxidative degradation of methyl ketones. II. Chemical pathway for degradation of 2-tridecanone by *Pseudomonas multivorans* and *Pseudomonas aeruginosa*. J. Bacteriol. 96:1055–1064.
3. Forney, F. W., and A. J. Markovetz. 1969. An enzyme system for aliphatic methyl ketone oxidation. Biochem. Biophys. Res. Commun. 37:31–38.
4. Forney, F. W., A. J. Markovetz, and R. E. Kallio. 1967. Bacterial oxidation of 2-tridecanone to 1-undecanol. J. Bacteriol. 93:649–655.
5. Fredricks, K. M. 1967. Products of the oxidation of *n*-decane by *Pseudomonas aeruginosa* and *Mycobacterium rhodochrous*. Antonie van Leeuwenhoek 33:41–48.
6. Klein, D. A., J. A. Davis, and L. E. Casida, Jr. 1968. Oxidation of *n*-alkanes to ketones by an *Arthrobacter* species. Antonie van Leeuwenhoek 34:495–503.
7. Klein, D. A., and F. A. Henning. 1969. Role of alcoholic intermediates in formation of isomeric ketones from *n*-hexadecane by a soil *Arthrobacter*. Appl. Microbiol. 17: 676–681.
8. Lukins, H. B., and J. W. Foster. 1963. Methyl ketone metabolism in hydrocarbon-utilizing mycobacteria. J. Bacteriol. 85:1074–1087.
9. McKenna, E. J., and R. E. Kallio. 1965. The biology of hydrocarbons. Annu. Rev. Microbiol. 19:183–208.
10. Markovetz, A. J., M. J. Klug, and F. W. Forney. 1967. Oxidation of 1-tetradecene by *Pseudomonas aeruginosa*. J. Bacteriol. 93:1289–1293.
11. Vestal, J. R., and J. J. Perry. 1969. Divergent metabolic pathways for propane and propionate utilization by a soil isolate. J. Bacteriol. 99:216–221.